ENDOCRINOLOGY

THE BULLETIN OF
THE ASSOCIATION FOR THE STUDY
OF INTERNAL SECRETIONS

VOLUME 24 FEBRUARY, 1939 NUMBER 2

MAINTENANCE OF THE CORPUS LUTEUM AND INHIBITION OF PARTURITION IN THE RABBIT BY INJECTION OF ESTROGENIC HORMONE

GEORGE P. HECKEL AND WILLARD M. ALLEN

From the Department of Obstetrics and Gynecology, University of Rochester, School of Medicine and Dentistry
ROCHESTER, NEW YORK

We have previously shown (1) that the injection of estrogenic hormone, beginning on the 11th day of pseudopregnancy, in the rabbit prevents regression of the corpora lutea, and that corpora caused to persist by this means are functional. Such corpora on the 26th day after a sterile mating usually are indistinguishable from those of pregnancy of similar duration, and their functional activity is shown by the observation of the uterus and endometrium. The uterine muscle remains insensitive to pituitrin in vitro, and the endometrium, retaining some proliferation, takes on the appearance of that of the sterile horn of a unilateral pregnancy of the same age. Normally in the absence of the injections, the corpora become non-functional on the 16th to 17th day, the uterus promptly becomes sensitive to pituitrin, and the endometrium reverts to the unproliferated estrous condition.

The purpose of the present experiments was to determine whether injections of estrogenic hormone would have a similar effect on the corpora lutea of pregnancy. The factors concerned in parturition in the rabbit are not fully understood, but it is established that parturition can be prevented either by the introduction of new corpora lutea (2) or by the injection of progesterone (3, 4), and that premature delivery can be produced by removal of the corpora. It is natural to assume, therefore, that one of the requirements for parturition is that the corpora lutea become non-functional or at least that there is a waning of the progesterone level. In view of our observation that regression of the corpora at the end of pseudopregnancy may be prevented by the injection of estrogenic hormone, we have now studied the effect of similar injections beginning during the latter part of pregnancy and have found, as expected, that such treatment prevents regression of the corpora lutea and likewise delivery.

EXPERIMENTAL OBSERVATIONS

In a group of preliminary experiments (table 1) injections of estrogen were begun on the 19th, 20th, 21st or 23rd days of pregnancy and continued either until delivery had occurred or until the animal was killed at the conclusion of the experiment. The injections were made so that the dose gradually increased, the expectation being that this method of administration might yield an estrogen level sufficiently high to prevent regression of the corpora and hence parturition. The individual experiments show that in no case, where the maximum total daily dose (usually achieved in the last day of the injections) was 175 I.U. or less, was parturition prevented from taking place at about the usual time. Animals receiving doses larger than this had macerated fetuses when they were killed on the 32nd to 38th day. One of these, 106, was killed on the 36th day. The corpora lutea were large and

TABLE 1. EFFECT OF CONTINUED INJECTIONS OF ESTROGEN IN THE LAST THIRD OF PREGNANCY

Rabbit no.	Daily dose minimum	Daily dose maximum	Period of injection	Delivery Type	Delivery Day	Fetuses
	I.U.	I.U.	days			
43	2500	32,500	19–32	Undelivered	33	Macerated
80	50	650	20–30	Undelivered	33	Macerated
106	50	500	20–35	Undelivered	36	4 macerated
105	50	400	20–33	Spontaneous	34	6 macerated
104	40	280	20–34	Spontaneous	before 38	Not found
114	35	228	20–34	Spontaneous	35	Not found
103	30	195	20–34	Undelivered	35	4 macerated
105	75	175	23–32	Spontaneous	34	7 dead
121	25	162	20–31	Spontaneous	32	8 living
120	23	146	20–33	Spontaneous	34	5 living, 3 dead
115	56	146	21–31	Spontaneous	32	8 living
103	20	140	20–32	Spontaneous	33	10 living
93	5	105	20–31	Spontaneous	32	5 living
94	2.5	32.5	20–32	Spontaneous	33	9 living

All animals were injected with estradiol monobenzoate. The amount was gradually increased as term approached.

histologically appeared to be normal. Further, the uterus contained 4 macerated fetuses. This suggests, of course, that the injections had maintained the corpora and also had killed the fetuses. The main reason for presenting these preliminary experiments is to show that premature delivery does not occur even though a quantity of estrogen sufficient to kill the fetuses is given. This result is contrary to reports in the literature that estrone induces abortion in rabbits (5, 6). The reason for this difference will be brought out later in this paper.

The next group of experiments consists of 6 rabbits injected with estrogen beginning on the 27th day (table 2) and one beginning on the 28th day of pregnancy, each receiving from 150 to 500 I.U. daily. In each case this quantity caused death of the fetuses and continuation of pregnancy beyond the 32nd day, the usual time for delivery. Two delivered on the 34th day, one on the 35th, and two on the 39th. Delivery occurred in spite of continuation of the injections except in 136 in which cessation of injections on the 36th day was followed by delivery on the 39th day. The remaining two

animals were killed undelivered on the 37th day. In both, the corpora lutea had not regressed as they would have if estrogen had not been given and normal parturition had occurred on the 32nd day. From these results it seemed evident that the corpora were being maintained by the estrogen and that their continued function was the reason for delay in parturition. Consequently 6 more rabbits were given comparable doses of estrogen but the ovaries were removed on the 27th day at the beginning of the injections. These 6 all delivered spontaneously on the 29th, 30th, or 31st days, 4 of

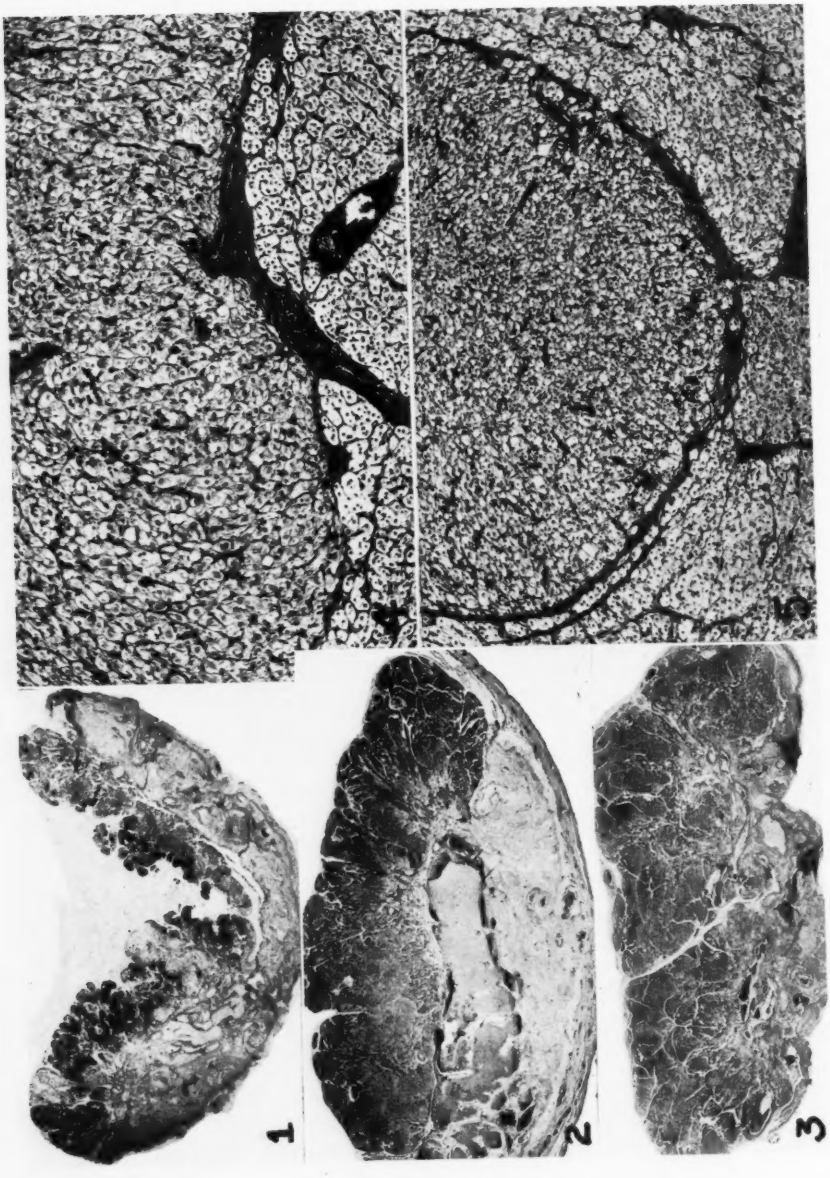
TABLE 2. COMPARISON OF THE EFFECT OF ESTROGEN GIVEN LATE IN PREGNANCY TO ANIMALS WITH OVARIES INTACT AND WITH OVARIES REMOVED

Rabbit no.	Daily dose per kg.	Daily dose total	Period of injection	Ovaries	Delivery Type	Delivery Day	Fetuses
	I.U.	I.U.	days				
149	45	150	27–34	Intact	Spontaneous	35	7 dead
137	57	200	27–36	Intact	Undelivered	37	9 macerated
147	61	200	28–38	Intact	Spontaneous	39	5 macerated
138	77	300	27–33	Intact	Spontaneous	34	7 macerated
136	110	400	27–36	Intact	Spontaneous	39	2 macerated
197[1]	119	500	27–34	Intact	Spontaneous	34	3 macerated
135	110	500	27–36	Intact	Undelivered	37	9 macerated
167	130	375	27–29	Removed 27th day	Spontaneous	30	6 macerated
170	130	450	27–28	Removed 27th day	Spontaneous	29	6 living
248	125	450	27–29	Removed 27th day	Spontaneous	29	2 living, 1 dead
180	116	500	27–28	Removed 27th day	Spontaneous	29	3 dead
					Spontaneous	30	2 dead, 1 living
181	120	550	27–29	Removed 27th day	Spontaneous	30	8 living
246	125	550	27–30	Removed 27th day	Spontaneous	30	4 macerated
					Spontaneous	31	7 macerated

[1] Injected with estrone. All others injected with estradiol monobenzoate.

them giving birth to some living fetuses, and two delivering macerated fetuses.

Another group of 10 animals was given estrogen beginning on the 29th day of pregnancy, the daily dose ranging from 100 to 1000 I.U. (table 3). None delivered on the 32nd day. All animals receiving between 100 and 375 I.U. daily, delivered spontaneously but in each case, excepting one which delivered on the 33rd day, delivery was delayed from 3 to 7 days. Two animals receiving doses of 500 and 1000 I.U. were killed undelivered on the 44th and 52nd days. The corpora lutea were examined in 4 animals. One (239) was killed on the 36th day when delivery occurred; 184, 237, and 182 were killed undelivered on the 35th, 44th, and 52nd days respectively. The corpora from these were of a size comparable to that of late pregnancy and histologically were essentially normal (fig. 4). Here again, to show that the corpora must be present for the inhibition of parturition to be obtained, 5 animals were castrated from one to 5 days after the injections were begun. Spontaneous delivery occurred within 48 hours despite continuation of injections of estrogen. This is in direct contrast to the animals in which similar injections were made but in which the ovaries, and hence the corpora lutea were intact. These controls are perhaps better than those of the group castrated on the 27th day because in this latter group the estrogen was given


1
2
3

time to exert an effect on the ovaries before they were removed, whereas in the previous group they were removed the day of the first injection.

In view of the finding that injections started as late as 3 days before term were effective in causing delay, a smaller series was studied in which injections were begun on the 30th and 31st day (table 4). These show several interesting things. Of 3 animals each given 500 I.U. daily beginning on the 31st day, only one delivered normally; one of the others delivered on the 41st day, and the third was killed undelivered on the 45th day. Incomplete delivery occasionally occurred. One, *212*, in which injections of 500 I.U. were begun on the 30th day, delivered one living and 3 dead fetuses on the

TABLE 3. EFFECT OF ESTROGEN BEGINNING ON THE 29TH DAY OF PREGNANCY

Rabbit no.	Daily dose per kg.	Daily dose total	Period of injection	Ovaries	Delivery Type	Delivery Day	Fetuses
	I.U.	I.U	days				
239	19	100	29–35	Intact	Spontaneous	36	9 macerated
328	28	100	29–32	Intact	Spontaneous	33	7 macerated
231	62	250	29–34	Intact	Spontaneous	35	7 macerated
233	62	250	29–36	Intact	Spontaneous	37	7 macerated
146	110	370	29–39	Intact	Spontaneous	39	4 macerated
232	120	375	29–37	Intact	Spontaneous	36	4 macerated
						39	1 macerated
184	122	500	29–34	Intact	Undelivered	35	5 macerated
182	128	500	29–51	Intact	Undelivered	52	4 macerated
198[1]	121	400	29–33	Intact	Spontaneous	34	7 macerated
237[1]	238	1000	29–43	Intact	Undelivered	44	11 macerated
228	121	350	29–34	Removed 34th day	Spontaneous	35	9 macerated
230	119	410	29–33	Removed 32nd day	Spontaneous	34	6 macerated
254	125	400	29–34	Removed 31st day	Spontaneous	33	1 macerated
					Died (autopsy)	34	4 macerated
264	119	500	29–31	Removed 30th day	Spontaneous	32	4 macerated
265	121	400	29–31	Removed 30th day	Spontaneous	32	6 macerated

[1] Injected with estrone. All others injected with estradiol monobenzoate.

The corpora lutea from *239*, *184*, *182*, and *237* were examined microscopically and found to be similar to those of a normal pregnancy.

31st day, but failed to deliver 5 more. On the 52nd day a laparotomy was made and both ovaries, which contained large pink corpora lutea, were removed. Death occurred two days later for reasons which were not apparent at autopsy. In this animal one horn had been completely evacuated on the 31st day while 5 fetuses, all dead, had been retained in the other horn. Another animal, *219*, receiving 1950 I.U. daily, delivered two living fetuses on the 32nd day, but failed to deliver the majority of the litter. On the 44th day the ovaries, containing large corpora lutea, were removed. Delivery failed to occur following removal of the ovaries, although a nest was made

Fig. 1. PLACENTA FROM 253 REMOVED FROM THE UTERUS ON THE 28TH DAY OF PREGNANCY. This animal received 1000 I.U. of estrone daily from the 24th to the 27th day of pregnancy. The fetal portion is much thinner than normal, and shows considerable necrosis. Compare with 2 and 3. ×3.

Fig. 2. NORMAL PLACENTA ON THE 27TH DAY OF PREGNANCY ATTACHED TO UTERINE WALL (226). ×3.

Fig. 3. NORMAL PLACENTA DELIVERED ON THE 32ND DAY OF PREGNANCY (258). ×3.

Fig. 4. CORPUS LUTEUM FROM 237 OBTAINED ON 44TH DAY OF PREGNANCY. The corpora had been maintained and parturition prevented by the injection of 1000 I.U. of estrone daily from the 29th to 44th days. ×40.

Fig. 5. CORPUS LUTEUM FROM A NORMAL RABBIT OBTAINED FOUR DAYS POSTPARTUM, THAT IS, ON THE 37TH DAY AFTER MATING (136). Note that the lutein cells are much smaller than those shown in fig. 4. ×40.

TABLE 4. EFFECT OF ESTROGEN BEGINNING ON THE 30TH AND 31ST DAY OF PREGNANCY

Rabbit no.	Daily dose per kg.	Daily dose total	Period of injection	Ovaries	Delivery Type	Delivery Day	Fetuses
	I.U.	I.U.	days				
213	125	400	30–36	Removed 35th day	Spontaneous	37	6 macerated
212	133	500	30–53	Removed 52nd day	Partial "	31	1 living, 3 dead
				Died 54th day	Undelivered	54	5 macerated
219	500	1950	30–49	Removed 44th day	Partial	32	2 living
					Undelivered	200	
218	500	1875	30–34	Intact	Spontaneous	35	7 macerated
159	130	500	31–41	Intact	Spontaneous	41	Not found
154	148	500	31–41	Intact	Undelivered	45	2 macerated
169	148	500	31–32	Intact	Spontaneous	33	10 living

All animals injected with estradiol monobenzoate.

The ovaries from *212*, *219*, *218*, *159* and *154* were examined on the 52nd 44th, 35th, 42nd and 45th days respectively and in each case the corpora lutea appeared like those of normal pregnancy before delivery.

two days after castration. It is interesting to note that a nest was previously made on the 32nd day when the two living fetuses were born. At the time of writing the animal has retained these fetuses in utero for 200 days and appears otherwise normal.

The foregoing experiments show also that in every case, where a significant delay in parturition was obtained or where parturition failed to occur, the fetuses were dead. In table 5 are listed several animals in which observations were made to determine the length of time between the beginning of injections and death of the fetuses. Three animals, killed 23, 27, and 27½ hours after a single injection of estradiol monobenzoate, were found to have dead fetuses in utero. Two others killed 21½ hours after injection also had dead fetuses. Inspection of the abdomen of one of these showed fetal movements 18½ hours after injection. The fetuses were removed immediately after killing the mothers in all cases and in none were there any spontaneous fetal movements. Some of the fetuses could be caused to move the limbs or gasp by stimulation, but respirations could not be established. Normal fetuses, of course, when removed in this manner on the 27th day are very active.

TABLE 5. EFFECT OF ESTROGEN LATE IN PREGNANCY ON THE FETUSES

Rabbit no.	Daily dose per kg.	Daily dose total	Days of injection	Time of observation after 1st injection	Type of observation	Fetuses
	I.U.	I.U.				
148	110	310	27–31	5 days	Autopsy	8 macerated
227	121	375	27–29	3 days	Autopsy	6 macerated
220	59	200	28	27½ hr.	Autopsy	9 dead
229	120	350	29	27 hr.	Autopsy	8 dead
244	63	270	27	23 hr.	Autopsy	9 dead
234	120	560	27	18½ hr.	Inspection	Active
				21½ hr.	Autopsy	2 dead
235	120	520	27	18½ hr.	Inspection	Inactive
				21½ hr.	Autopsy	8 dead or moribund

All animals injected with estradiol monobenzoate.

The exact cause for the fetal death has not been determined. In animals killed approximately 24 hours after the first injection the placentae are attached and appear to contain a normal amount of blood. Sections through such placentae show nothing which we can say with certainty is abnormal. Placentae of animals killed 3 or 4 days after the beginning of injections show striking changes (fig. 1). They are usually still attached, but the fetal portion is much thinner than normal, brownish grey in color, and necrotic. Microscopically there is only slight degeneration of the decidua, but marked degenerative changes are noted in the entire fetal portion. More study of the changes taking place within 24 hours of the first injection will have to be made before the cause for fetal death can be determined.

A final group of experiments were carried out to confirm the observation by others that estrogen induces abortion in rabbits, and to show that when it does occur under these conditions it is associated with regression of the

TABLE 6. EFFECTS OF ESTRONE ON THE FETUSES AND ON THE CORPORA LUTEA IN THE LATTER PART OF PREGNANCY

Rabbit no.	Estrone daily	Days injected	Remarks Parturition	Fetuses	Size of lutein cells (cross section)	Day corpora obtained
	I.U.				sq. mm. $\times 10^{-6}$	
201	2750	17, 18	Spontaneous 22nd day	Not found	534; 508; 542[1]	23
241	1000	24	Spontaneous 28th day	2 macerated	347; 347; 378	29
243	1000	24, 25	Spontaneous 28th day	Not found	378; 488; 443	28
252	1000	24	Spontaneous 28th day	2 dead	484; 539; 509 Avg. 458	28
240	1000	24–28	Undelivered 29th day	7 macerated	821; 803; 778	29
245	1000	24–28	Undelivered 29th day	7 living	783; 780; 845	29
250	1000	24–28	Undelivered 29th day	6 macerated	754; 832; 834	29
253	1000	24–27	Undelivered 28th day	7 macerated	725; 704; 782 Avg. 787	28
167	0		Normal pregnancy		778; 668; 743	27
170	0		Normal pregnancy		663; 793; 682	27
180	0		Normal pregnancy		782; 783; 857	27
181	0		Normal pregnancy		673; 778; 856	27
246	0		Normal pregnancy		636; 556; 685 Avg. 729	27

[1] Each figure represents the average of 10 cells.

corpora lutea. Raymond (5) has shown that the injection of a total of 240 to 480 I.U. of estrone on the 22nd and 23rd days of pregnancy is followed by delivery in about 4 days. In 6 animals so treated by him premature delivery occurred in two on the 26th day and in two on the 27th day. One animal delivered dead featuses, not macerated, on the 32nd day and the last was killed undelivered on the 28th day when living fetuses were found in the uterus. Robinson, Datnow, and Jeffcoate (6) obtained similar results. Estrogen given much earlier in pregnancy causes resorption of the embryos and need not be discussed here since it has no bearing on the mechanism of parturition.

In table 6 are shown the results obtained when estrone was given for a limited period only, as in the reports cited above. Two animals injected on the 24th (241, 252) and one on the 24th and 25th days (243) delivered on the 28th day. One animal (201) injected on the 17th and 18th days probably delivered on the 22nd day since fetuses were palpable on that day, but not on the next, and when autopsy was performed, only a placenta was found in

the uterus. The fetuses apparently had been delivered and eaten in the meantime. These results are essentially the same as those already recorded in the literature. In contrast to them, however, are the results obtained in a control group which received estrone daily (*240*, *245*, *250*, *253*). These received 1000 I.U. daily beginning on the 24th day. Three were killed on the 29th day, one day after abortion had already occurred in the 3 animals getting the same daily dose for only one or two days (*241*, *243*, *252*). In these the fetuses were dead, but one killed on the 28th day had living fetuses in utero. This is the only exception in all the experiments carried out in which doses of this order failed to kill the fetuses. In addition to these 3 animals can be cited the first 7 animals listed in table 1 in which the amount of estrogen given was always enough to kill the fetuses and yet premature delivery did not occur.

The explanation for the failure to deliver, even though the fetuses are killed, in the group where injections were continued, was found when the corpora were examined. In this group they appeared healthy, whereas in the group receiving injections for only one or two days they appeared smaller and paler. This difference was borne out when microscopic sections were studied. To make the difference even more convincing, however, the size of individual lutein cells was measured with a calibrated ocular micrometer. The scheme of measuring was as follows. In a single field under the oil immersion lens all of the lutein cells were measured in which the nucleus was clearly visible, then a second field, and so on until 30 different cells had been measured. Measurements were made simply by recording the greatest length and greatest width, and then computing the surface area assuming the shape to be elliptical. This method approximates the true area only, but for comparative purposes it is sufficiently accurate. Measurements were made in this manner of each of the 4 animals receiving estrone for one or two days and in which premature delivery occurred (*201*, *241*, *243*, *252*), of each of the 4 animals in which the injections were continued (*240*, *245*, *250*, *253*), and in 5 animals on the 27th day of normal pregnancy (table 6). The measurements show that in each animal in the first group, that is, those in which delivery occurred, the cells are all smaller than in the group getting estrone daily, even though the fetuses were killed in both, and also that the second group had cells as large as those of normal pregnancy. There is considerable variation in the size of the cells but nevertheless the results are consistent. All of the measurements (average of 10 cells) in the first group, without exception, are smaller than similar measurements in either the second or third group.

It is apparent from this last study that when estrone is given for one or two days, delivery of dead fetuses occurs a few days later, and this is associated with evidence of regressive changes in the corpora lutea, whereas if the estrone is continued, such regressive changes do not occur even though the fetuses are killed. When estrone is continued the corpora are maintained and hence delivery of the dead fetuses is prevented.

DISCUSSION

The results obtained show that estrogenic hormone is not an abortifacient in the ordinary sense, that is, it does not induce abortion because of any pri-

mary effect on the uterus. When it is given for a few days in the latter third of pregnancy, it is followed a few days after stopping the injections by premature delivery of dead fetuses. If the estrogen caused this effect by increasing the irritability of the uterus as is suggested by Robson (7) in his theory regarding the causes for parturition, then continuation of the injections should be even more effective than single injections. Such, however, is not the case for when injections are continued, the fetuses are killed the same as from single injections, but they are retained in utero. This seemingly contradictory result is readily explained. When single injections are given (or when injections are given for a few days and then discontinued) the fetuses are killed and extensive degenerative changes take place in the fetal portion of the placenta. This signifies that any functional activity of the placenta probably ceases. When it is recalled that Knaus (8) and Klein (9) have already shown that the products of conception (probably the placenta) are essential for persistance of the corpora lutea during the second half of pregnancy in the rabbit, it seems probable that in this type of experiment the placenta can no longer function and that delivery results because the corpora have begun to regress. This explanation is further supported by the observation that the cells of the corpora are really smaller than normal when premature delivery occurs under these circumstances. On the other hand, if injections of estrogen are continued, the fetuses are killed and degenerative changes occur in the placenta just as when single injections are made, but the corpora lutea appear healthy (table 6). The difference, then, is that in one group the corpora regress and in the other they do not; in the former delivery takes place, in the latter dead fetuses are retained in utero. This interpretation can obviously be extended to indicate that the placenta causes the corpora to persist under normal conditions by virtue of production of estrogenic hormones.

The major significance of the experiments, however, probably lies in the observation that the injection of estrogenic hormone, beginning even as late as one day before delivery ordinarily occurs, will cause the corpora lutea to remain functional, and thereby prevent parturition, presumably because of the continued capacity of the corpora for producing progesterone.

This may have considerable influence on theories regarding the onset of labor. It has been suggested by Robson (7) that the gradually increasing sensitivity of the rabbit's uterus to pituitrin which is observed during pregnancy is due to a gradually increasing amount of estrogenic hormone in the animal. There are good reasons for believing this. Estrogen increases the sensitivity of the uterus in castrated animals. In the latter part of pregnancy, the sterile horn of a unilateral pregnancy grows considerably and becomes more sensitive to pituitrin and at the same time there is some growth of the mammary glands. These are changes which may be presumed to be due to an increase in the amount of estrogen present. If this were the only factor responsible for the onset of labor, however, one would not expect that a further increase in the amount present, obtained by the injection of estrogen as in our experiments, would in any way delay parturition. The opposite would, in fact, be expected. When it is remembered that removal of the ovaries

after the 20th day is followed by premature labor, and that the induction of new corpora near term or the injection of progesterone prevent labor, it seems evident that regression of the corpora lutea also is of paramount importance. Any theory attempting to explain the cause for parturition must therefore include an explanation for the retrogression of the corpora lutea. It is obvious that, under the experimental conditions examined in the present report, any attempt to artificially raise the estrogen level prevents the decline of the corpora. This is true also in pseudopregnancy (1). A theory calling for a gradually increasing estrogen level does not agree with the idea that the corpora must decline. Another explanation suggests itself which does explain, but not necessarily correctly, all of the observed phenomena. If the estrogen level rises in the second half of pregnancy, and considerable evidence can be marshalled to support this, it may be supposed to remain at a high level practically until term. A decline a day or so before delivery would presumably be followed by decline in the activity of the corpora lutea, and this in turn by parturition. This simple theory can be used to explain all the experimental observations. Artificially maintaining a high progesterone level prevents parturition but does not prevent retrogression of the corpora (3, 4). Artificially maintaining a high estrogen level prevents decline of the corpora and indirectly through maintaining the progesterone level, prevents parturition.

If this explanation for the hormonal changes at parturition is the correct one, a reason should be found for the supposed falling of the estrogen level. This in turn calls for an explanation of the source of estrogen during pregnancy. We have now been able to maintain pregnancy to term with crystalline progesterone in animals castrated on the 11th day (unpublished observations). In these the sterile horn shows typical late pregnancy changes, the mammary glands grow, and milk is secreted just as in normal pregnancy. Furthermore, when the injections are stopped on the 28th day, delivery occurs in an essentially normal manner on the 31st or 32nd day. Such animals are deprived of all estrogen after the 11th day except that which is produced as a result of the pregnancy. This experiment shows that the ovaries produce nothing which is essential for the maintenance of pregnancy other than progesterone, and that, if estrogen is produced during pregnancy, it probably arises in the placenta. The supposed decline in the amount of estrogen produced as term is approached may then be considered to be due to a gradual ageing of the placenta which makes it unable to continue the production of estrogen. As a result of this the corpora are no longer supported and parturition results.

The above theory must be qualified and restricted to the animal in which the observations were made, the rabbit. It can not be transferred in its present form to man. It is well known that pregnancy can continue in women in a normal manner after both ovaries are removed providing oöphorectomy is not performed too early in pregnancy. This does not necessarily mean, however, that progesterone is not essential in the latter part of pregnancy. The pregnandiol output is high even late in pregnancy when the ovaries are not

necessary so it is probable that progesterone is being made by the placenta, this organ having taken over the function of the corpus luteum.

The question of the effect of the size of the fetuses on the induction of parturition has for the most part been answered by the experiments of Snyder (2) in which postmature fetuses were obtained when parturition was postponed by introducing new corpora into the ovaries. Under such circumstances the fetuses get much larger than normal and yet delivery does not occur. In the experiments recorded in this paper, the injection of estrogen kills the fetuses so that the distending mass does not continue to increase in size, yet delivery follows removal of the corpora lutea. The size of the fetuses per se would appear to have little or no part in causing the onset of labor. It is rather the state of the uterus itself, and this seems to be dependent on the proper balance of the ovarian hormones.

The inference from the experiments recorded is that parturition always occurs because of retrogession of the corpora. This is not proven for all cases by histologic studies since many animals were delivered several days past term even though injections of estrogen were continued. The corpora from these animals are prolonged. They do not show changes which are typical of retrogression, as seen 3 or 4 days after normal delivery. This, of course, may be construed as evidence that the decline of the corpus luteum has nothing to do with parturition since in those animals delayed parturition occurred with fairly normal looking corpora in the ovaries. This is not a valid objection to the interpretation placed on the present experiments, however, since parturition occurs also in the normal rabbit with healthy appearing corpora in the ovaries. The evidence in favor of a waning of the corpora as a cause for delivery does not rest on histologic retrogression but on the experiments already mentioned in which increasing the progesterone level in the animal either by injection of progesterone or by introduction of new corpora in the ovaries is found to delay parturition.

Another question also is of interest. Does parturition always occur when the corpora lutea are removed or after they have begun to regress? This can be answered negatively. Two animals were given single injections of 50,000 I.U. each of estradiol monobenzoate, one on the 20th and the other on the 27th day. In neither did parturition occur but when they were killed on the 36th and 37th days respectively, macerated fetuses were present in utero. Examination of the ovaries showed very small, white corpora which were in an advanced state of retrogression. In these delivery did not occur even though the corpora were obviously non-functional. Another animal (219) in which parturition had been prevented by estrogen was castrated on the 44th day. A nest was built the next day but delivery did not occur. In these two different types of experiment some disturbance of the uterine muscle apparently occurred, which prevented its proper function. In all animals in which delivery was delayed beyond the 39th day, the uterine muscle showed severe degenerative changes, in some being almost completely dead. This no doubt explains the inability to deliver after removal of the ovaries on the 44th day and later. It does not, of course, explain the original delay since re-

moval of the ovaries at the 30th to 34th days (table 3) was always followed by delivery.

SUMMARY

Parturition in the rabbit is delayed by the continued injection of estrogenic hormone in the latter part of pregnancy. The ovaries must be present for this result to be obtained. In animals in which delivery has been postponed by this means the corpora lutea are maintained and their continued activity is shown to be the cause for the delay in parturition.

Parturition is not delayed if injections of estrogen are not continued. Isolated injections in the latter part of pregnancy are followed by delivery of dead fetuses within 4 days. Under these circumstances the corpora lutea are not maintained. Cells of such corpora, by actual measurement, are smaller than those from corpora of normal pregnancy or from animals in which fetuses have been killed by estrogenic hormone but in which delivery has not occurred because of continuation of the injections. Doses of estrogenic hormone sufficient to delay parturition always cause death of the fetuses. A single injection on the 27th, 28th, or 29th day of pregnancy usually results in death of the fetuses within 27 hours.

REFERENCES

1. Allen, W. M., and G. P. Heckel: *Science* 84: 161. 1936.
2. Snyder, F. F.: *Bull. Johns Hopkins Hosp.* 54: 1. 1934.
3. Kopf, A. K., and M. E. Davis: *Am. J. Obst. and Gyn.* 34: 26. 1937.
4. Heckel, G. P., and W. M. Allen: *Am. J. Physiol.*, 119: 330. 1937.
5. Raymond, R.: Sur l'antagonisme fonctionnel entre les hormones ovariennes. Thèse Médecine Alger. 1934.
6. Robinson, A. L., M. N. Datnow and T. N. A. Jeffcoate: *Brit. M. J.* 1: 749. 1935.
7. Robson, J. M.: *J. Physiol.*, 81: 372. 1934.
8. Knaus, H.: *Arch. f. Gynäk.* 151: 395. 1930.
9. Klein, M.: *Compt. rend. Soc. de Biol.* 113: 441. 1933.

INDUCTION OF MATING IN THE DOG WITH PREGNANCY URINE EXTRACT

J. H. LEATHEM AND J. A. MORRELL

From the Biological Laboratory, Princeton University, and Research Laboratory, E. R. Squibb & Sons

PRINCETON AND NEW BRUNSWICK, NEW JERSEY

INDUCTION of typical estrus in the dog with pregnancy urine extract has been previously reported. Gaebler (1) was able to induce estrus in 1 of 5 dogs tested and Mathieu (2) also reported 1 successful case, but neither the criterion of typical estrus nor mating nor the duration of the estrous period was recorded. Although vulval swelling and, in some cases, bleeding generally resulted with this hormone another investigator was unable to induce estrus even by prolonged injections (3). Reiss and Langendorf (4) reported the production of cystic follicles and some corpora lutea as the result of injections of a pregnancy urine extract. They noted in some cases a considerable increase in ovarian size. Ito et al. (5) reported only the production of follicles. In our previous experiments on the dog, pregnancy urine extract was used only to produce ovulation after estrus had been induced by other extracts (6, 7). Therefore the effect of pregnancy urine extract (PU) alone was employed in this investigation to allow a comparison with the estrus induced with menopause urine extract (MUE) as well as with normal estrus.

METHODS AND RESULTS

Fifteen mature female dogs in the anestrous phase of their cycle were used for these experiments. Injections of pregnancy urine extract, follutein (E. R. Squibb & Sons), were made subcutaneously in daily dosages of 10, 25 or 50 R.U. per kilogram of body weight. Careful observations as to vulval swelling, hemorrhagic discharge and estrus as determined by mating were made. Vaginal smears were taken at regular intervals. The ovaries, uterus and a part of the vagina were removed from all dogs for histological study at different times after the injection of varying amounts of the hormone.

Attempts to Produce Mating (Estrus). Eleven dogs were injected until mating was induced. Vulval swelling resulted in every case regardless of dosage, and was first evident between the 5th and 8th days after the start of injections. In practically every case the swelling reached what was considered a maximal response. Hemorrhagic discharge, indicative of proestrous, was observed in only 3 cases. The only pronounced bleeding occurred in 2 of the 3 dogs on the largest dosage. The other case exhibited only slight bleeding on the dose level of 25 R.U. per kilo. A period of 3 to 9 days elapsed between the time of first vulval swelling until the time of mating. The dura-

tion of mating was 8 to 12 days or comparable in duration to that of normal estrus (table 1).

Vaginal smears were taken at regular intervals but failed to exhibit a true correspondence to the phases of the normal cycle. One dog, 5, which had delivered pups 3 weeks prior to the injection period, mated on the 8th day after the start of injections at which time the smear had shown no change from the anestrous type. In other cases the smear became partially cornified during proestrous but returned to an anestrous type during the

TABLE 1. EFFECTS OF AN EXTRACT OF PREGNANCY URINE, FOLLUTEIN, ON THE ESTROUS REACTIONS OF THE DOG

Dog	Weight	Period injected	Total R.U. injected	Beginning vulva swelling	Bleeding	Mating time from 1st inj.	Remarks
Dogs receiving 10 R.U. per kilogram							
	kg.	days		day	days	days	killed
1	8.6	13	1300	7th	—	16–26	5th day after mating ended
3	14.0	11	1540	8th	—	14–25	21st day after mating ended
7	10.5	8	800	5th	—	13–23	6th day after mating ended
14	9.4	9	900	8th	little	13–	5th day of mating
Dogs receiving 25 R.U. per kilogram							
4	10.5	11	2750	5th	—	13–23	End of mating
5	10.0	7	1750	5th	—	8–20	11th day after mating ended
9	12.3	8	2400	8th	1	12–23	6th day after mating ended
10	11.8	11	3300	6th	—	14–	5th day of mating
Dogs receiving 50 R.U. per kilogram							
2	9.1	11	4950	5th	8	13–	2nd day of mating
11	9.0	8	3600	5th	4	10–20	3rd day after mating ended
8	6.9	7	2450	5th	—	12–20	10th day after mating ended
15	8.4	4	1600	—	—	—	5th day of exp.
6	10.1	6	3000	—	—	—	8th day of exp.
Castrate Dogs							
12	9.2	—	—	—	—	—	25th day of exp., control
13	8.1	15	6000	—	—	—	23rd day of exp.

time the dog accepted the male. In one instance (dog 11) the smear remained cornified during mating and 2 other dogs, 10, 14, had also shown a cornified smear up to the time they were killed relatively soon after exhibiting the mating reaction. The pronounced increase in leucocytes which is a typical indication of the end of estrus or the beginning of metestrous was not observed with these dogs. In all cases there was a rapid return to an anestrous smear which is typical for this animal.

It was noted that neither the occurrence nor non-occurrence of bleeding nor the appearance of completely cornified smears were reliable indices of willingness to copulate with the male.

Effects on Vagina and Uterus. The vaginae of those dogs which had a cornified smear during estrus exhibited a cornified layer of cells. However the vaginal mucosa was not hypertrophied to as marked an extent as in

normal estrus, the squamous epithelium being 2 to 8 cells in thickness instead of 12 to 20. All other dogs showed the typical anestrous epithelium.

The 2 dogs, 6, 15, that were killed, before the appearance of external changes, failed to show any uterine hypertrophy. Four dogs were killed at different intervals during mating to allow a comparison of the uterus with that of normal estrus. In every case hypertrophy of the stroma and myometrium was evident. On the 2nd day of mating the endometrium was only slightly developed with a slight increase of the crypts around the lumen but little, if any, increase in the basal glands. After 5 days of mating (dog 10) the endometrium was further progressed but was comparable to a late proestrous type of the normal cycle. The crypts were not deep, and while the basal glands were increased, they were small and showed no evidence of coiling. Another animal, 14, killed after the same number of matings showed an endometrial development that was comparable to an early estrous condition (fig. 1, 2). The endometrium of a dog in mid-estrus of a normal cycle shows more hypertrophy and greater increased glandular development.

In early pseudopregnancy there is a marked increase in glandular complexity in the endometrium in both normal estrus and estrus induced with MUE (6). Seven dogs of this series were killed after the end of the mating phase for comparison with uteri of normal metestrous. Three days after cessation of mating the endometrium was of an early estrous development (fig. 3). Three days later no further hypertrophy was observed. The crypts were not deep and the basal ones were small. By the 11th day definite signs of regression were observed, although development never exceeded the early estrous type. Ten days later the endometrium was comparable to the anestrous type with a large lumen, absence of crypts and scattered small basal glands.

Unlike normal estrus and estrus induced with MUE, pregnancy urine extract induced mating in the dog which was not followed by a pseudopregnant period as indicated by the endometrium.

Two dogs were ovariectomized on the same day. One served as a control whereas the other was injected with 50 R.U. per kg. to determine what influence the extract might have on the uterus and vagina directly. Injections were begun 12 days after operation. Although this dog received 15 injections in 18 days or a total of 6000 R.U. no change in the smears or any external indications of estrus resulted. The endometrium had regressed.

Effects on the Ovary. Previous investigators reported a large increase in the weight and size of the ovary following injections of this extract (4, 5). Ito et al. (5) reported that the ovary increased to five times its normal weight. During mating the ovaries were found to be enlarged but the largest single ovary obtained in these experiments was 1.6 gm. This weight is only slightly heavier than a normal estrous ovary which has been found to weigh 1.3 gm., but is an increase over the anestrous ovary which weighs about 0.5 gm.

Two dogs, 6, 15, on the 50 R.U. per kg. dosage were killed after 4 and 6 injections, at which time no external changes were evident, in order to ob-

serve the effect on the ovary under these conditions. After 4 injections there was a slight increase in size of a few follicles whereas after 2 additional injections (dog 6) there was a definite increase in the amount of follicle stimulation.

In the remaining dogs of this series the ovaries were normal in appear-

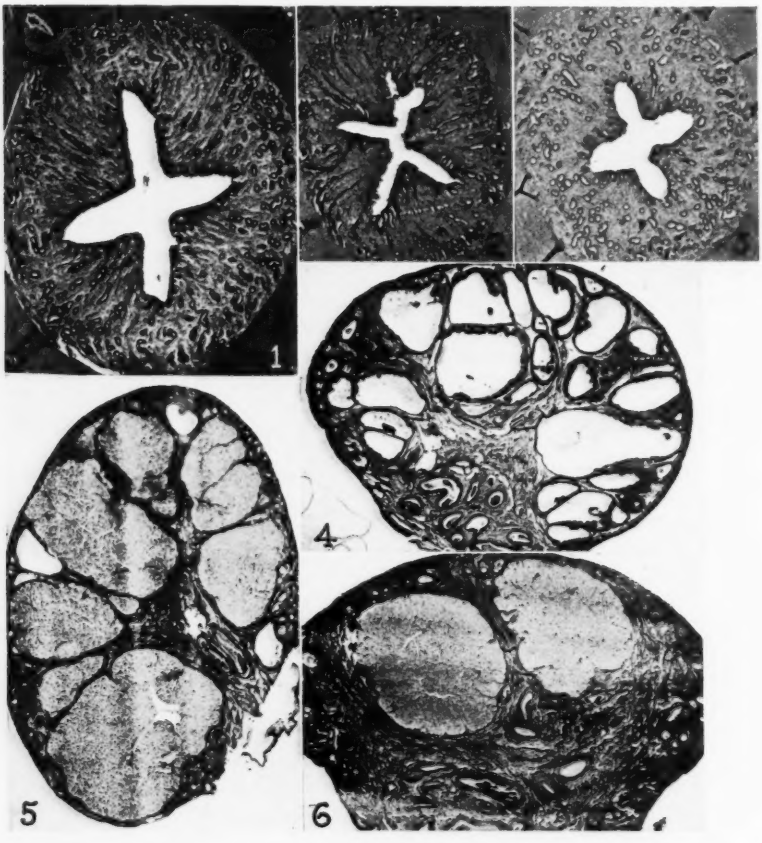


Fig. 1. Normal early estrus endometrium. ×6. Fig. 2. Endometrium on 5th day of induced mating. ×6. Fig. 3. Endometrium on 3rd day after mating stopped. ×6. Fig. 4. Ovary during induced mating showing follicle stimulation. ×4. Fig. 5. Ovary at end of induced mating showing corpora lutea. ×5. Fig. 6. Ovary 10 days after induced mating showing regressed corpora lutea. ×16.

ance in that they showed no signs of the excessive follicle stimulation such as was observed with MUE (6). Ovulation points were not observed in any case nor was there any histological evidence to indicate that ovulation had taken place. On the 2nd day of mating (dog 2) the ovary exhibited only follicle stimulation with little or no indication of luteinization. The ovary was a network of small and medium sized follicles, a stimulation quite differ-

ent from that observed with MUE where a smaller number of follicles were stimulated to a greater extent. In this case none of the follicles approached the size of a normal ovulating follicle (fig. 4). Ova were always present and generally free in the antrium. The greatest amount of follicle stimulation was observed with a dog, *14*, on the lowest dosage and killed the 5th day of mating. Many follicles were stimulated and in a few instances the follicles were of large size. Indications of luteinization were also observed in some follicles and in general the ova were free in the antrium regardless of the size of the follicle. Another dog, *10*, also killed on the 5th day of mating but on a higher dosage exhibited an ovary in which small corpora were found but follicle stimulation to medium sized follicles was most predominant. It was of interest to note in these cases that virtually all the follicles had their ova free from the granulosa and resembled a corona radiata formation. In normal estrus the corona radiata does not form until just prior to ovulation, but the follicles resulting from the injection of follutein, in nearly every case, did not approach the size of an ovulating follicle.

In one case (dog *4*) killed as soon as mating stopped the ovary was still of estrus size. Compact corpora lutea were observed which in some sections occupied a large part of the ovarian tissue (fig. 5). Some medium sized follicles were also observed in other sections.

At different intervals after mating stopped the ovaries were smaller in size and weight. Most of them approached anestrous size even during the first 10 days of this period. On the 3rd day after mating stopped the ovary contained only a few small corpora and a few small follicles. However, another dog, *1*, killed 2 days later had ovaries composed almost entirely of compact corpora. At least some of the corpora were definitely atretic since the degenerating ovum was observed in the follicle. Two dogs were killed on the 6th day of this period. One had regressed corpora in the periphery of the ovary, but not enough to occupy half the ovarian tissue. The other animal lacked residual corpora. Of the 4 dogs that were killed at virtually the same time in this period, the dog on the highest dosage had the least amount of corpora formation. By the 10th day only small regressed corpora were evident (fig. 6), and on the 21st day the ovary compared favorably with one at the end of pseudopregnancy or in early anestrum.

DISCUSSION

The results of these experiments indicated that mating may be induced in the mature female dog with pregnancy urine extract. Gaebler (1) successfully induced estrus in 1 of 5 dogs by a single large injection. In these experiments it was found that smaller daily injections induced mating which lasted for the same time as that in the normal estrous cycle in every one of the 11 dogs tested. Vulval swelling occurred but proestrous bleeding was generally absent. Difference in daily dosage was not reflected in the mating response. The vaginal smears showed no direct correlation with the sexual behavior. Completely cornified smears were obtained in only a few cases but even then the development of the vaginal epithelium was much less than normal.

Previous investigators (4, 5) observed uterine hypertrophy and some effect on the mucosa but did not correlate the endometrial condition to normal estrus. Some hypertrophy of the uterus was observed but even after several successive days of copulation the endometrium was still only comparable to that of proestrous or the first day of mating in the normal cycle. This early estrous development was observed in a dog on the 3rd day after cessation of mating. A pseudopregnant endometrium did not develop. Regression proceeded from this early estrous type endometrium. After the 5th day following the stopping of mating regression was noted. It proceeded so rapidly that an anestrous endometrium was found on the 21st day.

Increase in the size of the ovary was observed but in only one case did the weight surpass the normal estrous ovary. Soon after mating stopped the ovary had returned to anestrous size. Indication of the secretion of estrone by the ovary was demonstrated by the changes in vagina and uterus. Since no effects were obtained on the castrate the extract did not contain estrone. Secretion of estrone as the result of PU injections has been reported for many animals but Engle (8) found that this extract checked estrone secretion in the monkey.

The presence of only follicles in the ovary as the result of PU injections has been reported for the dog (5) while only corpora lutea and cystic follicles have been observed by other investigators (4). Both ovarian conditions were exhibited in these experiments depending on the time elapsed after injections were discontinued and the time the dog was killed. Early in estrus the ovary was a network of medium and small sized follicles whereas luteinization was first observed only after several days of mating. It has been shown that hypophysectomized puppies will not react to PU injections despite a long injection period (9). Experiments on the hypophysectomized mature dog would be of importance. On the other hand the normal monkey shows no follicle stimulation with PU (8).

Luteinization is not directly proportional to the dosage administered. A dog on 10 R.U. per kilo dosage exhibited the most corpora whereas a dog on the 50 R.U. per kilo at the same period had little corpora and a few follicles. The atretic corpora formed were maintained for only a short period in comparison to corpora found after estrus induced by MUE (6). By the 21st day after mating ended the ovary resembled that found 60 days after normal estrus.

Bourg (10) found that luteinization of follicles with PU resulted in the development of a pseudopregnant endometrium in the cat. In the normal rat, Shelesnyak (11) has demonstrated by producing deciduomata that the corpora formed with this extract are functional. Results of the experiments reported here would indicate that the corpora lutea are not active since a pseudopregnant endometrium did not form under the conditions of these experiments. However since the amount of estrone secreted was apparently less than in normal estrus, this may have prevented a progesterone stimulation of the endometrium.

SUMMARY

Pregnancy urine extract was found to be capable of inducing some external manifestations of estrus in the normal dog, namely, vulval swelling and mating but proestrous bleeding was generally absent. Induction of mating was successful with mature dogs in anestrous in all of the 11 cases tested, whereas the castrate failed to respond. Follicular stimulation to small and medium sized follicles resulted and after injections were discontinued corpora lutea atretica formed. Some endometrial hypertrophy also resulted. The changes produced failed to duplicate the normal cycle because *a*), the vaginal smears did not correspond, *b*), the endometrium developed only to an early estrous state and then regressed, *c*), a pseudopregnant period did not follow mating as shown by the failure of a pseudopregnant endometrium to develop. Furthermore only a small amount of lutein tissue formed in most cases and was maintained for only a short period and finally *d*), ovulation did not occur.

The authors wish to express their appreciation to Edwin S. Olssan for his assistance during the experimental work.

REFERENCES

1. Gaebler, O. H.: *Endocrinology* 19: 63. 1935.
2. Mathieu, F.: *Compt. rend. Soc. de biol.* 113: 903. 1933.
3. Koch, W.: *München. Tier. Wchnschr.* 84: 551. 1933.
4. Reiss, M., and K. Langendorf: *Endokrinologie* 3: 161. 1929.
5. Ito, M., S. Hayatsu and F. Ueno: *Endokrinologie* 16: 311. 1935.
6. Leathem, J. H.: *Endocrinology* 22: 559. 1938.
7. Leathem, J. H., and J. A. Morrell: *Endocrinology* 23: 164. 1938.
8. Engle, E. T.: *Am. J. Physiol.* 106: 145. 1933; 108: 528. 1934.
9. Reichert, F. L., R. I. Pencharz, M. E. Simpson, K. Meyer and H. M. Evans: *Am. J. Physiol.* 100: 157. 1931.
10. Bourg, R.: *Compt. rend. Soc. de biol.* 108: 216. 1932; 111: 148. 1932.
11. Shelesnyak, M. C.: *Am. J. Physiol.* 104: 693. 1933.

PROTOCOLS

Dog 1 Mature. Anestrous. Injections started 2/28/38. In 14 days 13 daily injections of 100 R.U. given. On 3/6 vulva swelling. On 3/9 smear 50% cornified. On 3/15 mated, smear anestrous. End of estrus 3/26. Killed 3/30. Uterus hypertrophied. Ovaries anestrous size with small corpora.

Dog 2 Mature. Anestrous. Injections started 2/28/38. 11 daily injections of 450 R.U. given. On 3/4 vulva swelling and bleeding. On 3/12 bleeding stopped. Mated. Smear some cornified cells. Killed 3/14. Uterus hypertrophied. Ovaries 1.26 grams.

Dog 3 Mature. Anestrous. Injections started 3/18/38. In 13 days 11 daily injections of 140 R.U. given. On 3/25 vulval swelling. On 3/28 smear cornified. On 3/31 mated. End of estrous 4/11. Killed 5/2. Uterus hypertrophied. Ovaries small with few corpora on surface.

Dog 4 Mature. Anestrous. Injections started 3/2/38. In 12 days 11 daily injections of 250 R.U. given. On 3/6 vulval swelling. On 3/9 smear mostly cornified. On 3/14 mated. On 3/17 smear only epithelial cells. Killed 3/24. Uterus hypertrophied. Ovaries normal estrous size.

Dog 5 Mature. Anestrous. Delivered 3 pups 3/4/38. On 3/6 pups taken. Injections started 3/21. In 8 days 7 daily injections of 250 R.U. given. On 3/25 vulval swelling. On 3/28 mated; smear anestrous. End of estrus 4/9. Killed 4/20. Uterus hypertrophied. Ovaries anestrous size.

Dog 6 Mature. Anestrous. Injections started 3/18/38. 6 daily injections of 50 R.U. given. Killed 3/25. Uterus small. Ovaries anestrous size. No vulval swelling or smear change.

Dog 7 Mature. Anestrous. Injections started 3/21/38. In 10 days 8 daily injections of 100 R.U. given. On 3/25 vulval swelling. On 4/2 estrus. End of estrus 4/12. Sacrificed 4/18. Uterus hypertrophied. Ovaries anestrous.

Dog 8 Mature. Anestrous. Injections started 3/21/38. In 8 days 7 daily injections of 350 R.U. given. On 3/25 vulval swelling. On 4/1 mated. End of estrus 4/9. Killed 4/19. Uterus hypertrophied. Ovaries anestrous.

Dog 9 Mature. Anestrous. Injections started 3/21/38. In 9 days 8 daily injections of 300 R.U. given. On 3/28 vulval swelling and slight bleeding. On 3/29 no bleeding. On 4/1 estrus. End of estrus 4/11. Killed 4/18. Uterus and ovaries small.

Dog 10 Mature. Anestrous. Injections started 4/25/38. In 12 days 11 daily injections of 300 R.U. given. On 4/30 vulva swelling. On 5/8 mated. Killed 5/13. Uterus hypertrophied; ovaries small; smear cornified.

Dog 11 Mature. Anestrous. Injections started 4/25/38. In 9 days 8 daily injections of 450 R.U. given. On 4/29 vulval swelling. On 5/2 bleeding. On 5/4 mated; smear mostly cornified. On 5/6 bleeding stopped. On 5/13 leucocytes in smear End of estrus 5/14. Killed 5/17. Uterus hypertrophied. Ovaries anestrous size.

Dog 12 Mature. Anestrous. On 4/26/38 castrated. Killed 6/3.

Dog 13 Mature. Anestrous. On 4/26/38 castrated. Injections started 5/9. In 18 days 15 daily injections of 400 R.U. given. Killed 5/31. No effect.

Dog 14 Mature. Anestrous. Injections started 5/9/38. In 10 days 9 daily injections of 100 R.U. given. On 5/16 vulval swelling. On 5/19 smear cornified. On 5/21 mated. Killed 5/25. Uterus small. Ovaries 1.6 grams.

Dog 15 Mature. Anestrous. Injections started 5/9/38. 4 daily injections of 400 R.U. given. Killed 5/13. No external reactions.

THE BIOLOGICAL ASSAY OF THE MAMMOGENIC DUCT GROWTH FACTOR OF THE ANTERIOR PITUITARY[1,2]

A. A. LEWIS, C. W. TURNER AND E. T. GOMEZ
WITH THE TECHNICAL ASSISTANCE OF W. T. CARROLL
From the Department of Dairy Husbandry, Missouri Agricultural Experiment Station
COLUMBIA, MISSOURI

IT HAS BEEN established that the ovarian hormones are ineffective in stimulating mammary gland growth in hypophysectomized male guinea pigs, mice and rats, and in female rabbits, cats, ground squirrels and guinea pigs (1, 2, 3, 4, 5, 6, 7). That the pituitary was necessary for the action of estrogen upon the mammary gland was concluded by Selye et al. (8).

Contrary results, however, have been reported by several investigators (9, 10). Ruinen (10), was unable to secure localized growth of mammary glands with estrogen painted on the skin. A large number of experiments always gave equal development of all glands.

Injection of desiccated whole pituitary powder from non-pregnant sheep was ineffective in stimulating mammary gland growth in three hypophysectomized immature female guinea pigs (4). One hypophysectomized and one castrated male rat showed no mammary gland growth after 30 daily implants of normal adult rat pituitaries. A normal male guinea pig gave similar results. Two immature castrated female rats and a rabbit injected with fresh anterior lobe tissue from non-pregnant heifers also showed no response (11).

When pituitary donors, however, were given preliminary treatment with estrogen under suitable conditions, it has been found possible to grow extensive mammary duct systems in hypophysectomized male and castrated female guinea pigs (4, 11). Mammary gland growth was also successful in castrate female rabbits on injection of fresh, macerated and desiccated prehypophyseal tissue from animals in various stages of pregnancy. Mammary glands of castrate immature female rats injected with fresh prehypophyseal tissue from pregnant cattle also responded with growth of the mammary duct system and the beginning of the lobule-alveolar system.

Gomez and Turner (11) have postulated the presence of a new entity called mammogen in the pituitaries of estrogen treated and pregnant animals. The purpose of the present paper is to describe experiments having as their object the determination of the suitability of male albino mice as assay animals for the duct growth factor of mammogen.

[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 575.
[2] Aided in part by a grant from the International Cancer Research Foundation.

Normal Rudimentary Condition of the Male Mouse Gland. The mammary glands of the male albino mouse remain rudimentary throughout life (12). Gardner et al. (13) noted that the mammary rudiments removed from male mice from 12 to 15 days of age were as well developed as those removed from normal males under 100 days, over 200 days, and over 700 days old. Since the experiments, reported here, deal with the male mouse at about puberty (10 to 25 gm.) it seemed well to investigate the influence of this period on the glands. Examination of 117 normal control male mice has shown no rudiments consisting of more than a few thin primary ducts with perhaps a few short side stubs. These glands vary in length from 0.4 mm. to 5.0 mm. with an average of thoracic, sternal and abdominal pairs of 1.2 mm. Examples of such control glands are shown in fig. 1. That within this weight range, at least, sexual maturity has no influence upon the length of the glands, is shown by a coefficient of correlation based on 117 mice of -0.0035 ± 0.093 between the weight of these mice and the average length of the glands in the above 3 pairs.

METHOD

The technic used in the treatment of fresh pituitary preparations involved the maceration of 20- to 40-gm. portions of frozen anterior-pituitary tissue. Small amounts of water were added during the process and the fine material put through a 100-mesh sieve. Fibrous material remaining after thorough grinding in a mortar was squeezed in a gauze bag to remove all the moisture possible and then discarded. The macerated material was diluted with distilled water to a concentration of 500 mg. of fresh tissue per cc. of water and preserved by freezing. Dilution of materials for injection was made with sterile distilled water. The pH was adjusted to about neutral and a drop of phenol added as a preservative. All mouse injections were made subcutaneously and once daily. The technic for preparation of the mouse skins and examination of the glands has been described by Turner and Gomez (14), except that the use of potassium alum was omitted and the unmounted glands were examined under the dissecting microscope while differentiating in 70 per cent alcohol.

EXPERIMENTAL RESULTS

The Effects of Gonadotropic Hormones and Androgens on the Mammary Rudiments. Fresh anterior-lobe tissue which was presumably rich in gonadotropic hormone was injected. Therefore it seemed essential to investigate the effects of injection of such hormones on normal male mice. A number of investigators have shown that certain androgens and gonadotropic hormones will stimulate growth of the mammary glands of male rats (15, 16, 17, 18). Such glands, however, develop to a considerable extent in the normal animal. This is not the case with male mice. No work on the effect of androgens and gonadotropic hormones on the mammary glands of male mice has come to our attention. Two such experiments were conducted.

Injection was made of APL gonadotropic hormone extracted from urine (antophysin[3]) at the rate of 560 R.U. per mouse over a 14-day period. Five mice gave entirely negative results in mammary stimulation (table 1). Similar results were secured with 3 mice injected over the same period with a total of 280 R.U. per mouse.

[3] Antophysin furnished by the Winthrop Chemical Co., Inc.

A group of 6 male mice were injected subcutaneously with 0.1 cc. (0.1 mg.) of testosterone propionate (oreton[4]) in olive oil. A second group of 6 mice was injected with twice as much (0.2 mg.) daily. On the 8th, 16th and 25th days, two animals from each group were killed. Examination of the whole-mount preparation of the mammary glands of animals after 7 and 15 days' injection in both groups showed no evidence of stimulation of the rudiments (table 1). After 25 days, the glands of animals receiving 0.1 mg. of oreton daily showed slight arborization of the duct system as indicated by

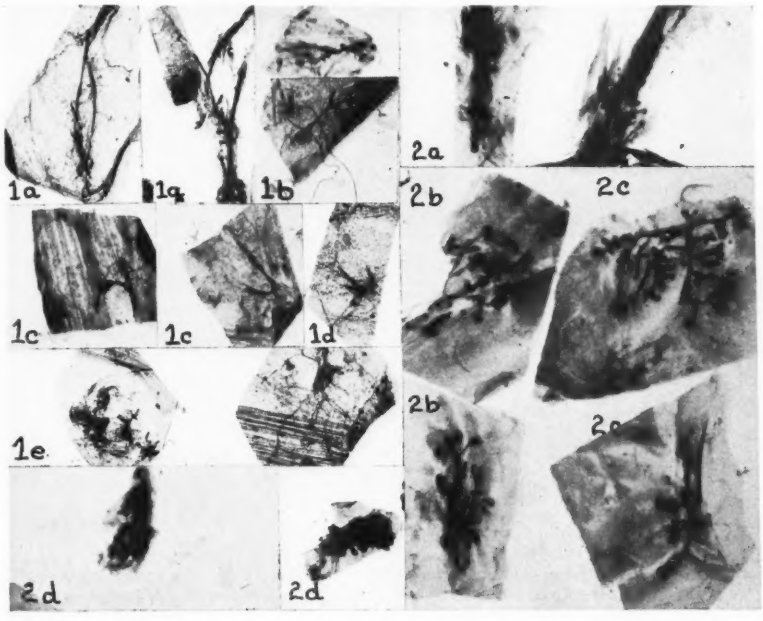


Fig. 1. EXAMPLES OF NORMAL CONTROL MAMMARY RUDIMENTS FROM MALE ALBINO MICE 0.4 mm. to 50.0 mm. in length. A. Cervical; B. Thoracic; C. Sternal; D. Abdominal; E. Inguinal. Fig. 2. MAMMARY GLANDS FROM A HEAVILY STIMULATED MALE ALBINO MOUSE INJECTED DURING 6 DAYS WITH 120 MG. OF FRESH PREHYPOPHYSEAL TISSUE FROM PREGNANT CATTLE. Even more difference in stimulation between glands on the same animal is often found. Usually some rudiments do not respond. A. Cervical; B. Thoracic; C. Sternal; D. Abdominal. (×6)

the presence of short lateral branches and distention of the distal ends of primary and secondary ducts. The animals receiving 0.2 mg. of oreton daily for 25 days showed further extension of the main and secondary ducts and lateral branches. The extent of this growth was similar to that obtained after the 10-day injection of theelin in male mice (12), and also like fig. 3.

It is evident from the above results that injection of gonadotropic hormone (antophysin) does not influence the growth of the glands following 14 daily injections with large dosage. Only upon injection of an androgen (oreton) for 25 days were definite signs of growth observed.

[4] Oreton furnished by the Schering Corporation.

Administration of Estrogen. Turner et al. (19) found that 2.5 R.U. of theelin injected daily for 20 days into castrate female rats gave good response in growth of the mammary duct system. Similarly 16.7 R.U. of theelin daily for 12 days in male rabbits gave considerable duct proliferation. Five R.U. of theelin daily for 15 days gave no response when injected into male mice. The same results were secured with 0.5 cc. of theelol. Gardner (13), however, was able to produce glands similar to those of fig. 4 with 2 R.U. of theelin per day for 15 days.

Turner and Gomez (14) found that 10 R.U. of theelin when injected daily for 20 to 30 days into adult albino male mice would cause extensive growth

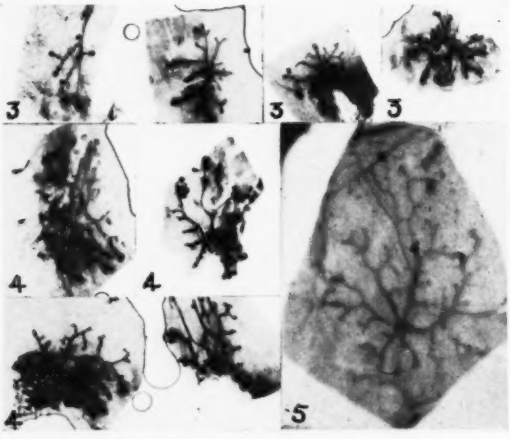


Fig. 3. EARLY STAGES OF MAMMARY GLAND GROWTH IN MALE ALBINO MICE UNDER THE STIMULATION OF MAMMOGEN FROM PREGNANT CATTLE PREHYPOPHYSIS. Large, dark staining end-buds, thickened ducts and proliferation of ducts are apparent. One such gland obtained from an animal is sufficient evidence of mammary growth stimulation. (×2.5). Fig. 4. LATER STAGES OF MAMMARY GROWTH STIMULATION IN MALE ALBINO MICE WITH GREATER PROLIFERATION OF DUCTS. Enlarged end-buds are not always in evidence in later stages. (×2.5). Fig. 5. A LARGE GLAND OBTAINED AFTER 10 DAYS INJECTION WITH 200 MG. TOTAL OF PREHYPOPHYSEAL TISSUE FROM PREGNANT CATTLE. Equivalent to a normal gland from any early estrous female mouse. (×6)

of the duct system. No attempt was made, however, in these experiments to determine the minimum amount of the estrogenic hormone which would cause mammary duct growth. Nor was there obtained the minimum time required to produce observable signs of growth stimulation.

In order to ascertain the dosage required and the response of pubertal male mice to mammary growth stimulation, benzoate of hydroxyestrin (progynon-B[5]) was injected into three groups of 5 mice, and one of 10, at the rate of 100 I.U. (20 R.U.) per day for 4, 6, 8 and 10 days. The total dosage of 400 to 1000 I.U. gave definite proliferation of glands. The response of the 10 mice to 600 I.U. injected in 6 days was good (fig. 3 and 4.) Seven of the mice showed definite signs of mammary growth stimulation. The effect appeared to be quite generalized, glands showing more uniform development than in positive results reported below on injection of prehypophyseal tissue from preg-

[5] Supplied by the Schering Corporation.

nant cattle. A 50 per cent response would require, evidently, somewhat less than 600 I.U. in 6 days.

Administration of Fresh Anterior Lobe Tissue from Pregnant Cattle. Daily injection of 20 mg. of fresh prehypophseal tissue from cows, pregnant less than 140 days, for 4, 7 and 10 days resulted in gradually increasing percentages of positive cases (25 to 100 per cent) as well as more heavily stimulated glands (fig. 3 and 4). Insufficient material was available to run a larger group of mice.

These experiments established that the mammary glands of male albino mice are normally very rudimentary during the pubertal stage and that growth stimulation can be obtained with estrogenic hormone and with pregnancy cattle prehypophysis. The following experiments therefore were conducted to determine a suitable assay technic using the male mouse.

Injection for 5, 7, and 9 days of 22.25 mg. per day of fresh anterior-lobe tissue from cattle of unknown stages of pregnancy[6] (table 1) gave results in definite signs of gland growth in 2 out of 4, 2 out of 4, and 3 out of 4 mice, respectively. One mouse given 200 mg. in 9 days showed a very strong response with glands comparing favorably with those of pubertal females of the same age (fig. 4).

The next experiment attempted to show the results of injecting twice the amount of material per day as in the previous experiment for a shorter time. Four mice injected for 3 days with 44.5 mg. per day of fresh pregnancy cattle prehypophysis showed no development over control glands. One more day of injection with 4 other mice gave, however, a 50-per-cent response. After 5 daily injections (223 mg. total) all 4 mice showed from one to several glands with definite signs of proliferation (fig. 3). These results for both 4 and 5 days were similar but the dosage required was 178 mg. for the former instead of 111 mg.

An attempt was made to compare the response of castrate mice to that of normal males. Two castrates were injected for 3 days with a total of 90 mg. of fresh pregnancy pituitary resulting in one animal with two glands showing slight proliferation. Seven castrates injected for 4 days with a total of 120 mg. of tissue gave two positive cases and one questionable. This compares favorably with the dosage of 178 mg. required for a 50-per-cent response with normal males. The 6-day injection of 180 mg., however, resulted in only one definitely stimulated mouse, and one questionable out of 4, whereas with normal males, 105 mg. have been found sufficient in 6 injections. These experiments demonstrate that castrate as well as normal male mice will respond to injection of pregnancy cattle prehypophysis.

Due to the negative results secured with injections of male sex hormone, except on long continued injection, and with gonadotropic hormone, the use of normal male mice seems justified for assay purposes. Since some question may remain, however, as to the supplementing effect of testicular hormones in the normal male mouse, it is suggested that castrates may be used, if desired, in their stead.

The response and dosage required on injection during the convenient

[6] Frozen pituitaries were supplied by Swift & Co., Kansas City, Mo.

TABLE 1. RESULTS OF INJECTION OF MALE ALBINO MICE WITH VARIOUS ENDOCRINE PREPARATIONS ON MAMMARY GLAND GROWTH

Preparation	No. of mice	Days injected	Dosage		Results[1]	Positive %
			Daily	Total		
Gonadotropic and Androgenic Hormones						
Ordinary gonadotropic hormone (antophysin)	5	14	40 R.U.	560 R.U.	0+, 5−	0
	3	14	20 R.U.	280 R.U.	0+, 3−	0
Testosterone propionate (oreton)	2	8	0.1 mg.	0.8 mg.	0+, 2−	0
	2	16	0.1 mg.	1.6 mg.	0+, 2−	0
	2	25	0.1 mg.	2.5 mg.	2+, 0−	100
	2	8	0.2 mg.	1.6 mg.	0+, 2−	0
	2	16	0.2 mg.	3.2 mg.	0+, 2−	0
	2	25	0.2 mg.	5.0 mg.	2+, 0−	100
Estrogenic Hormone						
Benzoate of hydroxyestrin (progynon-B)	5	4	100 I.U.	400 I.U.	2+, 3−	40
	10	6	100 I.U.	600 I.U.	7+, 3−	70
	5	8	100 I.U.	800 I.U.	4+, 1−	80
	5	10	100 I.U.	1000 I.U.	2+, 3−	40
Early-Pregnancy Cattle Anterior Lobes						
Fresh early pregnancy cattle anterior lobe, 17	4	4	20 mg.	80 mg.	1+, 3−	25
	4	7	20 mg.	140 mg.	2+, 2−	50
	4	10	20 mg.	200 mg.	4+	100
Fresh Anterior Lobes from Cattle of Unknown Stages of Pregnancy						
Fresh pregnancy cattle anterior lobe	4	5	22.25 mg.	111 mg.	2+, 2−	50
	4	7	22.25 mg.	156 mg.	2+, 2−	50
	4	9	22.25 mg.	200 mg.	3+, 1−	75
Fresh pregnancy cattle anterior lobe	4	3	44.5 mg.	133 mg.	0+, 4−	0
	4	4	44.5 mg.	178 mg.	2+, 2−	50
	4	5	44.5 mg.	223 mg.	4+, 0	100
Castrates: Fresh pregnancy cattle anterior lobe	2	3	30 mg.	90 mg.	1+, 1−	50
	7	4	30 mg.	120 mg.	2+, 4−, 1?	29
	4	6	30 mg.	180 mg.		
Fresh pregnancy cattle anterior lobe, lot 2	3	6		96.5 mg.	1+, 2−	33
	4	6		108 mg.	2+, 1−, 1?	50
	4	6		120 mg.	3+, 1−	75
	4	6		125 mg.	2+, 2−	50
	3	6		133 mg.	0+, 3−	0
Fresh pregnancy cattle anterior lobe, lot 1	4	6		94 mg.	1+, 2−, 1?	25
	10	6		105 mg.	5+, 5−	50
	4	6		115 mg.	2+, 2−	50

[1] Plus sign refers to definite signs of stimulation in one or more rudimentary glands of one animal; minus sign means absence of stimulation.

period of 6 days was then investigated. Four groups of 4 mice each were injected with 96.5, 108, 120, 125 and 133 mg., respectively, of fresh anterior lobe from pregnant cattle. The response, even with these small numbers, was well graded (table 1).

In a second experiment for the same period with another lot of pituitaries,

in which increasing dosage was given, a 50-per-cent response was obtained with 105 mg. (table 1). It is interesting to note that two different lots of mixed pregnancy pituitaries received at different times produced a 50-per-cent response with the same dosage.

The Proposed Assay Technic. As a result of these experiments the normal (10 to 25 gm.) male albino mouse seemed suitable for the development of a biological assay technic for at least the duct growth factor of mammogen, because of its small size, ready response, and the normal rudimentary condition of the glands, which do not respond to gonadotropic hormone.

Subcutaneous injection of fresh, macerated pregnancy anterior lobe tissue into the lumbar region of 10 to 25 gm. male mice once daily for a minimum of 4 days has been found to stimulate the mammary glands to development of thick ducts, numerous side branches and large, club-like dark staining end-buds. Often not all the glands on a skin will develop to the same extent. Those at the anterior end seem to respond most readily.

The total dosage needed to secure definite signs of development of at least one gland per skin can be reduced by injecting smaller amounts daily for 6 days. This is of advantage in assaying individual pituitaries. Six days' injection, killing on the seventh, is a convenient period. Because of variability in mouse response, it is recommended that definite signs of development be secured in 50 ± 10 per cent of 10 mice. Since the mice are rather susceptible to infection and may die as a result of the protein preparations used, it is advisable to start extra mice in each assay.

The repeatability of this assay technic is shown in table 2. The results

TABLE 2. THE REPEATABILITY OF THE RECOMMENDED ASSAY TECHNIC FOR THE DUCT GROWTH FACTOR, MAMMOGEN

Trial	Dosage	Positive	Negative	Percentage positive
1	105 mg. fresh macerated anterior pituitary lobe from pregnant cattle, injected during 6 days	4	6	40
2	Same.	7	3	70
3	Same.	5	4	56
4	Same.	4	6	40
5	Same.	6	3	67
6	Same.	4	5	44
		30	27	53

with 57 mice were well within the recommended range at 53 per cent positive. In 4 of the 6 trials, the results were also within the range. In the fifth trial with 9 mice living on completion of the assay, positive mice were 7 per cent over the range. The chances are that the 10th mouse would have reduced this error. Only in the second trial were the results definitely outside the range recommended.

SUMMARY

The use of male albino mice in the biological assay of the mammary duct growth factor of prehypophyses, mammogen, is reported. Large doses of gonadotropic hormone were entirely negative for mammary growth stimula-

tion as was male sex hormone for at least the first 16 days of injection. The injections of oreton were rather heavy and should surpass the male hormone which could be developed under stimulation of injections of fresh pituitary tissue. This question is to be further investigated.

The influence of varying daily dosages of estrogen and prehypophyseal tissue from pregnant cattle, and of length of injection period was studied. It was concluded that the convenient period of 6 days was satisfactory. The proposed mouse assay unit for the mammogenic duct growth factor is the total amount of tissue or extract required to produce definite signs of development in one or more glands of 50 ± 10 per cent of 10 male albino mice weighing 10 to 25 gm. Injection should be made once daily, subcutaneously, for 6 successive days, and the animal killed on the seventh.

The reproducibility of the assay method was shown by results obtained with 6 groups of mice. Four groups were within the proposed limit while one was definitely outside the limit. Twenty mice in a group would increase the accuracy of the results.

REFERENCES

1. Reece, R. P., C. W. Turner and R. T. Hill: *Proc. Soc. Exper. Biol. & Med.* 34: 204. 1936.
2. Lyons, W. R., and R. I. Pencharz: *Proc. Soc. Exper. Biol. & Med.* 33: 589. 1936.
3. Gomez, E. T., and C. W. Turner: *Proc. Soc. Exper. Biol. & Med.* 34: 320. 1936.
4. Gomez, E. T., and C. W. Turner: *Mo. Agr. Exper. Sta. Res. Bul.* 259: 20. 1937.
5. Gomez, E. T., C. W. Turner, W. U. Gardner and R. T. Hill: *Proc. Soc. Exper. Biol. & Med.* 36: 287. 1937.
6. Gomez, E. T., C. W. Turner and R. P. Reece: *Proc. Soc. Exper. Biol. & Med.* 36: 286. 1937.
7. Nelson, W. O., and C. E. Tobin: *Anat. Rec.* 67: 111 (suppl.). 1936.
8. Selye, H., J. B. Collip and D. L. Thomson: *Proc. Soc. Exper. Biol. & Med.* 32: 1377. 1935.
9. Freud, J., and S. E. De Jongh: *Acta Brev. Neerl.* 5: 47. 1935.
10. Ruinen, F. H.: *Acta Brev. Neerl.* 2: 161. 1932.
11. Gomez, E. T., and C. W. Turner: *Proc. Soc. Exper. Biol. & Med.* 37: 607, 1938. (Also unpublished data).
12. Turner, C. W., A. H. Frank, W. U. Gardner, A. B. Schultz and E. T. Gomez: *Anat. Rec.* 53: 321. 1932.
13. Gardner, W. U., A. W. Diddle, E. Allen and L. C. Strong: *Anat. Rec.* 60: 457. 1934.
14. Turner, C. W., and E. T. Gomez: *Mo. Agr. Exper. Sta. Res. Bul.* 182: 21. 1933.
15. Astwood, E. B., C. F. Geschickter and E. O. Rausch: *Am. J. Anat.* 61: 373. 1937.
16. McEven, C. S., H. Selye and J. B. Collip: *Proc. Soc. Exper. Biol. & Med.* 34: 56. 1936.
17. McEven, C. S., H. Selye and J. B. Collip: *Proc. Soc. Exper. Biol. & Med.* 36: 213. 1937.
18. Nelson, W. O., and T. F. Gallagher: *Science* 84: 230. 1936.
19. Turner, C. W., and E. T. Gomez: *Mo. Agr. Exper. Sta. Res. Bul.* 206: 6. 1934.

COPULATORY ACTIVITY IN ADULT MALE RATS FOLLOWING CASTRATION AND INJECTIONS OF TESTOSTERONE PROPIONATE[1]

CALVIN P. STONE
From the Department of Psychology, Stanford University
STANFORD UNIVERSITY, CALIFORNIA

THIS STUDY is concerned with the loss of sexual aggressiveness in adult male rats following castration and the restoration of sexual activity by subcutaneous injections of testosterone propionate.

Steinach (1) in 1894 observed that the male rat may copulate as late as 1 year following total castration (1 case). Since that time, many investigators have verified the fact of copulatory ability in man and the lower vertebrates following castration (2). In 1927 Stone (3) studied the copulatory activity of 45 male rats that had been castrated at the age of 90 days. These males were given short tests for copulatory activity at intervals of from 4 to 6 days until they were sexually inactive for a period of approximately 30 days. Considering the group as a whole, 33 per cent had ceased to copulate before the end of the first month following castration; 45 per cent, before the end of the second month; 57 per cent, the third; 74 per cent, the fourth; 79 per cent, the fifth; and 91 per cent, the sixth. A hit-or-miss relationship between night of testing and copulatory activity was an outstanding feature of the terminal phase of potency in most of the castrates. Thus, a male might be relatively inactive for two or three test nights and then display a considerable degree of aggressiveness on the next occasion of testing.

No attempt was made in the foregoing study to describe the waning of potency in quantitative terms or to restore potency by gonadal hormone. Nissen's study (4) has successfully dealt with both aspects of the problem. Ball (5) found a gradual waning of sexual activity following castration of pubescent rats and likewise a gradual waxing of sexual aggressiveness with injections of progynon-B (Schering). Some of the males reached a level of copulatory activity closely approximating their precastration level. Only 2 of the 6 castrated animals, however, exhibited the ejaculatory reflex. Successful restorations of potency by injections of synthetic preparations of the gonadal hormone in man and laboratory animals have been reported by Hamilton (6, 7), Shapiro (8), and Miller, Hubert and Hamilton (9).

PROCEDURE

Five normal and 11 castrated males were used in this experiment. All

[1] The study was supported by a fund from the Committee for Research on Sex Problems, National Research Council, and by a gift of 600 mg. of testosterone propionate from Ciba Pharmaceutical Products, Inc. Grateful acknowledgement is made to Mr. Max Lund, graduate student, who assisted in the observational program of this study.

were 10 months of age at the outset of the study and from 18 to 20 months old at the time the tests were completed. They were segregated from females after weaning except when needed for siring litters. Then females were left in their cages for short periods of time. For approximately 3 weeks after their 9th month all were given brief activation tests with receptive females every 3 or 4 nights to assure us that they were continually sexually potent. Two or three copulations but no ejaculations were allowed. Then followed a week of sexual deprivation preparatory to the preliminary test of sexual aggressiveness.

The preliminary test consisted of two 15-minute periods, separated by an interval of 20 minutes. Each male was alone in its home cage and a receptive female was put with it. The receptive female had been tested with other males to ascertain whether she was highly active and not inclined to fight. During the test, the pair of animals was continually under the surveillance of a trained observer, and records of the number of copulatory acts and ejaculations were made. Mountings accompanied by manual palpation and pelvic movements but without intromissions were called 'attempts.' During the first part of the study, which had to do with the waning of potency following castration, the number of attempts was not recorded. This regrettable omission was corrected in the tests having to do with reactivation.

On the days following the preliminary tests, 11 of the males were castrated. Then, 5 days later, and thereafter at 6- or 7-day intervals, standard tests similar to the preliminary tests were given. The total period of elapsed time between the date of castration and the last quantitative test during this phase of the study was 41 days. Using the data from the preliminary tests, the males were distributed into control and castration groups so as to make the means of copulatory and ejaculatory frequency approximately equal. The loss of one male following castration increased the means of the castrated group slightly, but to so small a degree that the original differences may be neglected.

RESULTS

Waning of Sexual Activity During Six Weeks Following Castration. Table 1 presents the copulation and ejaculation data of the controls and castrates. Possibly surgical shock and tenderness of the unhealed scrotum are partly responsible for the decline in copulatory and ejaculatory frequency of the castrates in test 1. That these factors would be influential beyond the first test, however, is highly improbable. Rats *7-c* and *8-c* were outstanding for the persistence of high frequencies of copulations and ejaculations. A few cases of this kind were expected on the basis of the earlier study (7). However, their frequencies are considerably lower than would have been expected from normal rats. The vigorous performance of *15-c* on the fourth test is enigmatical in the light of his previous and his later record of activity. Although other instances of this kind had been encountered in our work with castrates, there has been no case with a frequency as outstanding as this.

In addition to reduction of frequency there were marked differences in the temporal aspects of the sexual aggressiveness of the castrates. Some were

active at once when put with the females and completed two or three copulatory acts in close succession, but thereafter would be inactive for a long period. Other rats displayed no interest in the females at the outset but for no apparent reason later on suddenly bestirred themselves and were still active when the tests were terminated. Because of these differences in tem-

TABLE 1. NUMBERS OF COPULATIONS AND EJACULATIONS BY NORMAL AND CASTRATED MALES IN A 30-MINUTE PRELIMINARY TEST AND 6 TESTS OF SIMILAR DURATION AFTER THE EXPERIMENTAL GROUP WAS CASTRATED

Rat number	Prelim-inary		Tests, at 6-day intervals, after castration												Totals	
	I		II		III		IV		V		VI			
	c[3]	e[4]	c	e	c	e	c	e	c	e	c	e	c	e
1-n[1]	19	3	37	4	24	3	23	3	23	2	23	4	27	3	176	22
2-n	17	3	38	4	26	4	22	4	17	4	26	3	38	4	184	26
3-n	19	2	29	4	21	2	22	2	26	2	22	2	22	3	161	17
4-n	19	3	12	2	23	2	27	3	24	3	26	3	23	4	154	20
5-n	18	1	12	1	8	1	19	1	10	1	12	1	16	1	95	7
6-c[2]	18	2	6	0	3	0	1	0	0	0	0	0	0	0	28	2
7-c	29	3	11	1	14	1	15	1	14	1	12	0	0	0	95	7
8-c	19	2	7	1	4	0	11	1	10	1	13	1	4	0	68	6
9-c	18	2	11	2	10	1	2	0	0	0	0	0	0	0	41	5
10-c	17	2	10	1	5	0	7	0	6	0	0	0	0	0	45	3
11-c	28	4	15	1	6	0	8	0	0	0	0	0	0	0	57	5
12-c	19	2	9	1	0	0	0	0	0	0	0	0	0	0	28	3
13-c	15	1	6	0	3	0	6	0	0	0	4	0	0	0	34	1
14-c	25	4	15	2	6	0	0	0	1	0	6	0	4	0	57	6
15-c	20	3	9	1	4	0	8	0	34	1	1	0	0	0	76	5
Mean copul's n	18.4		25.6		20.4		22.6		20.0		21.8		25.2			
Mean copul's c	20.8		9.9		5.5		5.8		6.5		3.6		0.8			
Mean ejacul's n	2.4		3.0		2.4		2.6		2.4		2.6		3.0			
Mean ejacul's c	2.5		1.0		0.2		0.2		0.3		0.1		0.0			

[1] n=normal rat.
[2] c=castrate rat.
[3] c=copulations.
[4] e=ejaculations.

poral relationships, it would be necessary to use somewhat longer tests than used here to describe accurately the waning of sexual aggressiveness in the post-castration period. There is no reason to believe, however, that with longer tests the differences found between normal and castrated males, in general, would have been minimized. On the contrary, in most instances longer tests would have magnified the differences shown in table 1.

Period of Unrecorded Sexual Activity. The foregoing tests ended in the latter part of June. During the month of July attempts were made to activate these males by the use of theelin (5). The results of these tests were equivocal. In some of the animals there appeared to be an augmentation of sexual aggressiveness that was associated with the injections of theelin; in others this was not the case. The greatest degree of augmentation of activity was obtained in the cases of 6-*c* and 7-*c* which were still quite active in the fifth test following castration. Details of this phase of the experiment need not concern us further in this report.

Then, for a period of approximately 8 weeks the normals and the castrates were put into breeding cages with adult females. From time to time the females were changed to assure that non-pregnant animals were always present in the cages. At the end of this period, which we assumed (on the basis of our 1927 study) would be sufficiently long to eliminate potency in the castrates, an 8-day period of sexual abstention was imposed on all of the males preparatory to having them resume tests for sexual activity. This period of abstention lasted from October 1 to October 7.[2]

All of the normal males were tested once between October 8 and 20. The number of copulations and ejaculations for each male during a 15-minute test yielded the data given in table 2. This number roughly corresponds to results obtained in the first half of the previous tests and is typical of our nor-

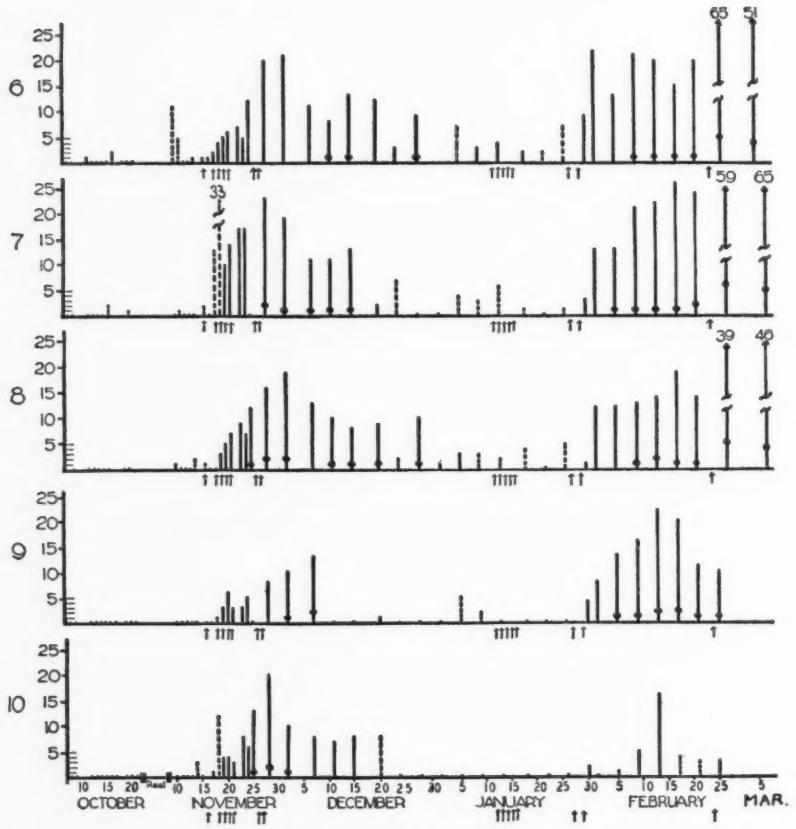


Fig. 1. (*Continued on next page*)

[2] Calendar dates are given in order that the reader hereafter may locate each essential event on the time line of fig. 1.

Fig. 1. (*Continued*)

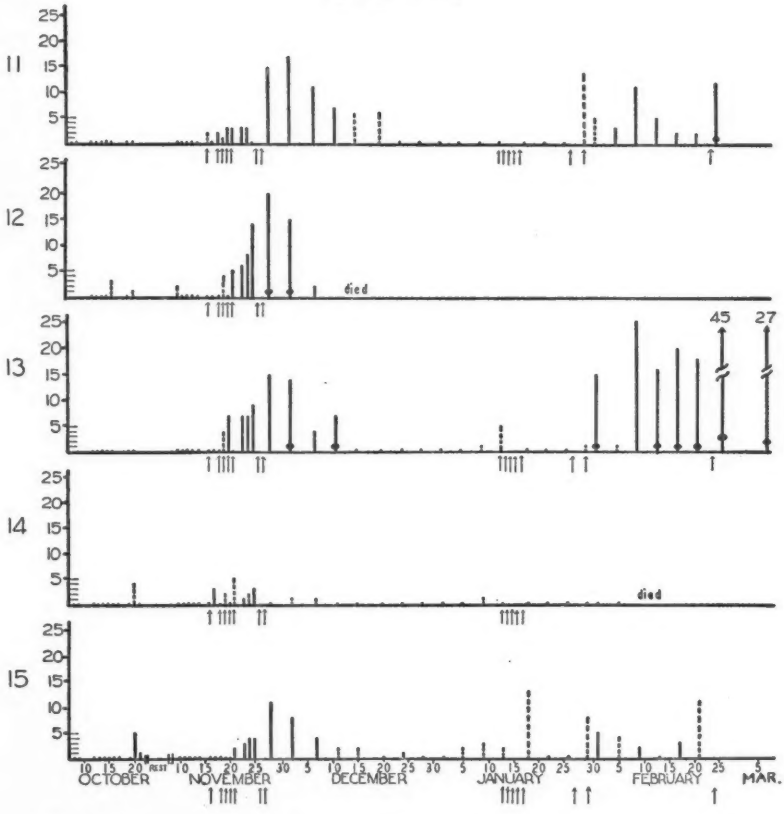


Fig. 1. COPULATORY AND EJACULATORY ACTIVITY IN CASTRATED MALE RATS. The animals' numbers appear at the extreme left. Columns, erected on base line, indicate the night when tests were made. Heights of broken line columns denote frequency of copulatory attempts; height of solid lines, frequency of copulations. Dots are placed on the columns at the proper height to indicate the frequency of ejaculations. Arrows at base lines indicate time of injections. On January 12 to 16 control injections of sesame oil were administered; all of the others consisted of 1.25 mg. of testosterone propionate dissolved in ¼ cc. sesame oil.

mal males of this age (10). Subsequently the normal males were used as testers for the females that were to be used with the castrated males during the following months. Further demonstration of their potency seemed unnecessary as our experience with animals similarly tested allows us to assume that they would have continued to yield results similar to those shown in table 2.

From the 8th to the 21st of October each of the castrated males was given a 15-minute test for copulatory activity. Rat 6-c was the only male that copulated at all and only a few attempts were made by any of the others. Then followed a short period of no testing (Oct. 22 to Nov. 9) in which the castrates did not have access to females. On the night of November 10,

TABLE 2. NUMBER OF COPULATIONS AND EJACULATIONS MADE BY NORMAL MALES WHEN TESTED BETWEEN THE DATES OF OCTOBER 8 AND 20. TESTS WERE 15 MINUTES LONG

Rat number									
1-n		2-n		3-n		4-n		5-n	
c	e	c	e	c	e	c	e	c	e
14	2	12	2	10	1	12	2	8	1

tests were resumed. Rat 6-c continued to display mild sexual activity and a few of the others made one or two copulatory attempts during the test.

Reactivation of the Castrates by Male Hormone. On November 16, approximately 8 hours before the time of testing, each castrate was given a subcutaneous injection of testosterone propionate. The dosage consisted of 1.25 mg. of this hormone dissolved in ¼ cc. of sesame oil. The same amount was injected on the subsequent dates indicated by the arrow at the base line of each record shown in fig. 1. The columns denoting frequency of copulation and ejaculation are erected at the proper points on the base line to indicate the calendar dates on which the tests were given. The position of the dot on the column gives the frequency of ejaculations.

A consideration of the data graphically represented in fig. 1 suggests that some of the animals may have been slightly stimulated as early as the first day following the injection (cf. 10-c, 11-c, 14-c), but that cannot be claimed for the majority of the group. On the second day after the first injection (8 hours after the second) 6-c, 7-c, 10-c, and 11-c appeared to be slightly more aggresive than would have been expected from the previous tests. And on the days immediately following, unmistakable evidence of the stimulating effects of the hormone were displayed by most of the castrates. In this phase of the experiment it was quite possible that daily exercise of the copulatory mechanism by the more vigorous animals caused some reduction of potency. Because of this possibility, a space of 4 or 5 days was interspersed between test trials after the waxing phase of potency had been observed. Beginning with the night of November 29, two 15-minute periods with an interval of 20 minutes between were given each male. These were similar in duration to the preliminary test and to the 6 post-castration tests.

The first ejaculation occurred on the 9th day after injections began. For unknown reasons, no ejaculations were elicited from 11-c and 15-c in this phase of the study although they were robust males. Rat 4, for which no instance of ejaculation was recorded, was not in good health. His weight had dropped from the precastration weight of 330 gm. to 225 gm., and much of the hair over the loin and hips had fallen out. The rigidity of his ribs resembled that of animals in an advanced stage of senility and his movements about the cage were similar to those of senile animals. Although he was not strictly comparable to the other castrates of the group, we kept him in the experiment nevertheless and treated him as the others were treated until the time of his death. Rat 20 appeared to be a healthy animal until a few days before his death from a respiratory disorder.

As shown in fig. 1, the last injection of this series was given on November 26. For approximately one and a half months tests were continued without further injections, to enable us to study the gradual waning of potency. In all cases ejaculations quickly ceased to appear and in most individuals copulatory frequency gradually waned. That with the passing of another month it would have reached the pre-injection level is highly probable. Apparently ejaculation appears later and disappears earlier than vigorous copulation in connection with testicular replacement therapy.

Control Injections of Sesame Oil. From the 12th to 16th of January control injections of ¼ cc. of sesame oil, the solvent for the testosterone propionate, were given. As far as could be determined, these injections had no augmenting effects on sexual aggressiveness. Animals that had been inactive in the immediately preceding tests remained inactive; those that were slightly aggressive showed no heightening of aggressiveness.

In marked contrast to the activity between January 13 and 26 is that following the injections of testosterone propionate on January 27 and 29. In all cases except rat 1 the return of copulatory activity was prompt. As in the tests following injections in November, vigorous and frequent copulation appeared before ejaculations. The cause or significance of this finding is not understood at the present time.

Four of the most active males of this group (6-c, 7-c, 8-c, 10-c) were subjected to tests of 3 consecutive hours' duration on February 26 and March 7. The results of these tests are given in fig. 1. For a group of 35 normal males the mean number of copulations in a 3-hour period was 60 and the mean number of ejaculations, 6.1. The records of these 4 males fall within the range of the middle 50 per cent of normal males, but it is improbable that they had reached quite the level that would have been reached had they been normal. The second 3-hour test which followed after an interval of 8 days yielded results for ejaculations slightly lower than those of the first test. In general, this would not be found with normal males. Presumably it denotes the waning effect of the last injection of male hormone which was administered on February 24.

Obstruction Tests. On March 25 and 26 eight of the animals were given a modified form of the Columbia University obstruction test. Nissen used this apparatus with castrates in 1927 at a time when testicular extracts, but not chemically pure testicular hormone, were available for use (4). He reported that:

> Injections of the lipoid fraction of testis-epididymis extract into castrated males are without effect. There is some indication of a facilitating influence on the sex drive of gonadectomized males by injections of the water-soluble residue of the testis-epididymis substance.

The obstruction apparatus used here and the methods of preparing the animals for the experiment are fully described in an earlier study on sexual drive by Stone, Tomilin, and Barker (10). Since these tests differ in several ways from those used by Nissen, we shall not attempt to compare our numerical results with his.

In preparation for this test each male had been given spaced injections of

1.25 mg. of testosterone propionate. The last three injections had fallen on the dates of February 24, March 15, and March 22. The castrates had not had opportunity for copulation after March 6. Before removing an animal from the home cage for the test, one copulation was allowed, by way of activating him under familiar conditions. Additional opportunity for copulation was allowed in the preliminary phases of the test and also during the 5-minute intermission between the two halves of the test proper. Occasionally a male copulated after crossing the grid despite the experimenter's intention to remove him before this could take place. Copulatory activity in the apparatus was permitted to give sexual meaning to the test situation.

Table 3 gives the number of crossings made by each castrate in the 10-minute periods of the obstruction test. Also, in this table are given the numbers of copulations and ejaculations by each male.

TABLE 3. RESULTS OF THE OBSTRUCTION TESTS. THE TEST CONSISTED OF TWO SUBTESTS OF 10 MINUTES EACH SEPARATED BY A FIVE-MINUTE INTERMISSION

Rat number	Number of crossings			Frequency	
	first 10′	second 10′	total 20′	copulations	ejaculations
6-c	20	26	46	13	0
7-c	2	10	12	14	1
8-c	7	2	9	13	2
9-c	3	10	13	13	2
10-c	0	0	0	1*	0
11-c	1	0	1	1*	0
14-c	0	10	10	10	1
15-c	1	1	2	12	1

* Copulated only in the home cage.

It is interesting to note that rats *10-c* and *11-c* which showed no copulatory aggressiveness in the apparatus also failed to give adequate evidence of sexual 'desire' by crossing the grid to reach the female. Most of the other males were sexually aggressive in the apparatus and also crossed the grid a number of times to reach the incentive. The fact that they copulated or made attempts when given an opportunity is our strongest inferential evidence for assuming that sexual 'desire' prompted their crossing the punishment grid. None of the males has a frequency score equal to the mean of the normal males previously studied (10), although that of *6-c* is well up in the distribution of normal animals. For 33 normal animals a mean of 64 was obtained; for *6-c* the frequency was 46. Since our previous study showed only a low degree of correlation between copulatory frequency and grid crossings, we did not expect a high degree of correlation between these measures for the castrates. Our data conform to this expectancy, although they are too few to justify any sweeping generalization on this point.

This phase of our study should be repeated with a larger number of castrates differing in sexual aggressiveness in order that the strength of sexual drive associated with testosterone propionate therapy may be more accurately described in the obstruction situation. Further systematic experi-

ments should be made in order to determine proper dosage and temporal spacing of injections.

Gross Appearance of the Reproductive Tracts. The normal controls and the castrates were sacrificed 3 days after the latter had completed the obstruction tests. At this time, it will be recalled, 6 of the castrated males had demonstrated sexual aggressiveness (table 3) and 2 were relatively inactive on the test night. Their reproductive tracts were carefully dissected out and

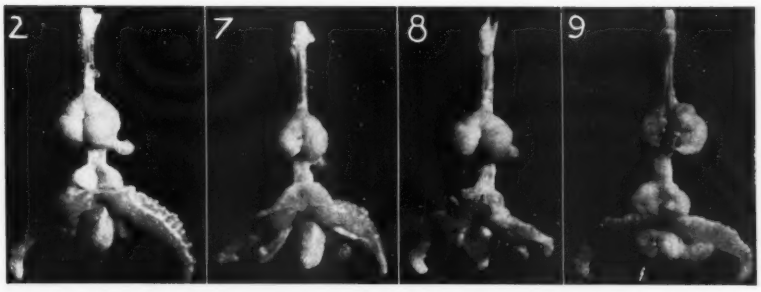


Fig. 2. ACCESSORY SEXUAL ORGANS OF A NORMAL MALE, 2, and 3 CASTRATES, 7, 8, 9. The organs were dissected out 3 days after last tests were made on the obstruction apparatus and while the castrates were still under the influence of the male hormone. The last injection was given seven days prior to killing the animals. ×0.6.

mounted in the manner shown in fig. 2. Inspection of the reproductive tracts of the normal and the castrated individuals led to the conclusion that in gross appearance they differed only in a few respects. As shown in fig. 2, one seminal vesicle of number 8 was very small and resembled the atrophic seminal vesicles of untreated castrates [cf. fig. 7 (3) p. 382]. In some instances the coagulatory glands were not as well filled with fluid as in the normal males. However, all had sufficient fluid to account for the vaginal plugs delivered by the castrates.

No histological examinations of any parts of the reproductive tracts were made. The studies of Moore and Price (11, 12) indicate that injections of testosterone propionate effectively restore and maintain the reproductive tract in castrated males.

SUMMARY

In this, as in previous studies of castrated male rats, sexual potency gradually waned, as measured by copulatory and ejaculatory frequency, until all of the castrates were totally inactive or only slightly active with receptive females.

Sexual activity was restored in all of the inactivated castrates by subcutaneous injections of testosterone propionate. Copulatory frequency in the most active castrates closely approached that of their precastration records and also the records of normal controls.

In all cases the ejaculatory reflex was slower in returning than the copulatory response. Also it was the first to go when injections were discontinued.

Sexual aggressiveness gradually waned after injections ceased, very much as it had done following castration of the males. Control injections of sesame oil, the solvent of the male hormone, did not augment the sexual activity of the castrates. But in most of the cases renewal of hormonal therapy speedily restored sexual aggressiveness to the high level previously reached after the first series of injections.

Obstruction tests were given to 8 of the castrates while still under the influence of the testosterone propionate. Although 6 of the castrates gave evidence of sexual drive, as measured by this test, only one of them displayed a degree of aggressiveness similar to that of typical normal males.

In gross appearance, the reproductive tracts of the castrates differed only slightly from those of the controls, the chief difference being a lesser amount of fluid in the coagulatory glands of the castrates.

REFERENCES

1. Steinach, E.: *Arch. f. ges. Physiol.* 56: 304. 1894.
2. Commins, W. D., and C. P. Stone: *Psychol. Bull.* 29: 493. 1932.
3. Stone, C. P.: *J. Comp. Psychol.* 7: 369. 1927.
4. Nissen, H.: *Genet. Psychol. Monog.* 5: 451. 1929.
5. Ball, J.: *J. Comp. Psychol.* 24: 137. 1937.
6. Hamilton, J. B.: *Endocrinology* 21: 649. 1937.
7. Hamilton, J. B.: *Endocrinology* 21: 744. 1937.
8. Shapiro, H. A.: *Nature* 139: 588. 1937.
9. Miller, N. E., G. Hubert and J. B. Hamilton: *Proc. Soc. Exp. Biol. & Med.* 38: 538. 1938.
10. Stone, C. P., M. I. Tomilin and R. G. Barker: *J. Comp. Psychol.* 19: 215. 1935.
11. Moore, C. R., and D. Price: *Endocrinology* 21: 313. 1937.
12. Moore, C. R., and D. Price: *Anat. Rec.* 71: 59. 1938.

THE EFFECT OF PROLONGED TESTOSTERONE PROPIONATE ADMINISTRATION ON THE IMMATURE AND ADULT FEMALE RAT

MILTON MAZER AND CHARLES MAZER
From the Departments of Gynecology and Medicine, Mt. Sinai Hospital
PHILADELPHIA, PENNSYLVANIA

THE SYNTHETIC ANDROGENS, testosterone and testosterone propionate, given over a relatively short period, have been found to stimulate the ovary of the normal rat. In adult rats, Korenchevsky et al. (1) observed a 29 per cent average increase in ovarian weight and an increase in the number and size of corpora lutea, as compared with controls, after daily hypodermic injections of 1.5 mg. of testosterone propionate for 21 days. Wolfe and Hamilton (2) likewise found moderately enlarged corpora lutea with daily injections for 10 days of 0.5 mg. of testosterone propionate, but only in those animals which were in estrus or metestrus at the start of the experiment. When treatment was begun in diestrus the corpora lutea varied in size but were generally no larger than those of controls. McKeown and Zuckerman (3) who administered daily doses of 0.2 mg. of testosterone propionate for 10 days to adult rats noted enlarged corpora lutea, whether the animals were in estrus, proestrus or diestrus at the start. In the immature rat, Salmon (4) found that single injections of 1 to 5 mg. of testosterone propionate or androstenediol caused follicle stimulation, with or without luteinization, and premature opening of the vagina within 60 to 192 hours after the injection. On the other hand McEuen and his associates (5), have reported that the administration of testosterone in 2 mg. doses daily for 23 days to immature rats inhibited the normal growth of the ovaries, as judged by weight.

Coincident with the ovarian stimulation described by Korenchevsky and his associates (1, 6) there was uterine and vaginal growth and suppression of estrus. The vaginal epithelium showed mucification similar to that seen in pregnancy. Paralleling the pregnancy-like vaginal changes were those in the uterus, the mucosa possessing "lace-like projections somewhat similar to those seen in the uterus in pregnancy." McKeown and Zuckerman (3) likewise found pregnancy-like changes in the uterus and, in one of 6 rats whose uteri were traumatized during the testosterone propionate treatment, they observed a "well-developed placentoma with typical decidual cells in rapid division" (pseudopregnancy).

From the foregoing review it may be seen that testosterone propionate given in effective doses over a period up to 21 days causes stimulation of the rat ovary and changes in the uterus and vagina similar to those seen in pregnancy. In view of these perhaps unanticipated effects, it seemed desirable to investigate the action of the substance in the rat when given over a prolonged period.

METHODS AND RESULTS

Immature Rats. Testosterone propionate,[1] 0.5 mg. in 0.2 cc. of sesame oil, was administered hypodermically 3 times weekly for 102 days from infancy to maturity to a group of 10 white rats whose initial weights were 30 to 40 gm. A group of 8 control animals received the same quantity of sesame oil without testosterone propionate for an equal length of time. Vaginal smears were studied daily. On the day after the last injection the animals were killed, the pituitary, ovaries, uterine horns and adrenals dissected free of extraneous tissue and weighed in the fresh state. The ovaries, uterus, and vagina were studied histologically.

The ovaries of the treated animals killed at full maturity had an average weight of only 10 mg., comparable to that of 22-day-old rats. The ovaries of the control animals averaged 45 mg. which is the usual ovarian weight for adult rats (table 1). Histological study revealed complete arrest of follicular development and an absence of corpora lutea (fig. 1 and 2). The uterine horns of the treated animals were grossly atrophic and only 47 per cent of the weight of control uterine horns (table 1). Microscopic examination showed atrophy of both mucosa and myometrium (fig. 3 and 4). In contrast to the control animals, all of which reached sexual maturity at the usual age, the treated animals never became sexually active and remained in diestrus throughout the experiment. Sections of the vagina of treated animals showed mucification of the epithelium. The pituitary glands of the treated animals were smaller and were only 58 per cent of the weight of the control pituitaries. The adrenals were also smaller, 70 per cent of the weight of the controls (table 1). Involution of the thymus occurred more rapidly in the treated animals.

TABLE 1. EFFECT OF PROLONGED TESTOSTERONE PROPIONATE ADMINISTRATION ON THE IMMATURE RAT

	Average Weight of Organs	
	Control Rats (8)	Treated Rats (10)
Body Weight at Autopsy (gm.)	149	145
Ovaries (mg.)	45	10
Uterine Horns (mg.)	386	178
Adrenals (mg.)	37	26
Pituitary (mg.)	9.0	5.2

It is noteworthy to observe that the testosterone propionate treatment over a period of 102 days caused no inhibition of growth, as determined by body weight (table 1), corroborating the finding of McEuen and his associates (5).

Adult Rats. Testosterone propionate, 0.5 mg. in 0.2 cc. of sesame oil, was administered hypodermically 3 times weekly for 62 to 68 days to a group of 20 adult rats. On the day following the last injection 15 of the animals were killed. The remaining 5 received no further treatment until autopsy 29 days later. A group of 6 control animals received the same quantity of

[1] The authors are indebted to Doctors Gregory Stragnell and Erwin Schwenk of the Schering Corporation for the generous supply of testosterone propionate (oreton) used in these experiments.

sesame oil without testosterone propionate for an equal length of time. Vaginal smears of all animals were studied daily. The autopsies were performed as described for the immature rats.

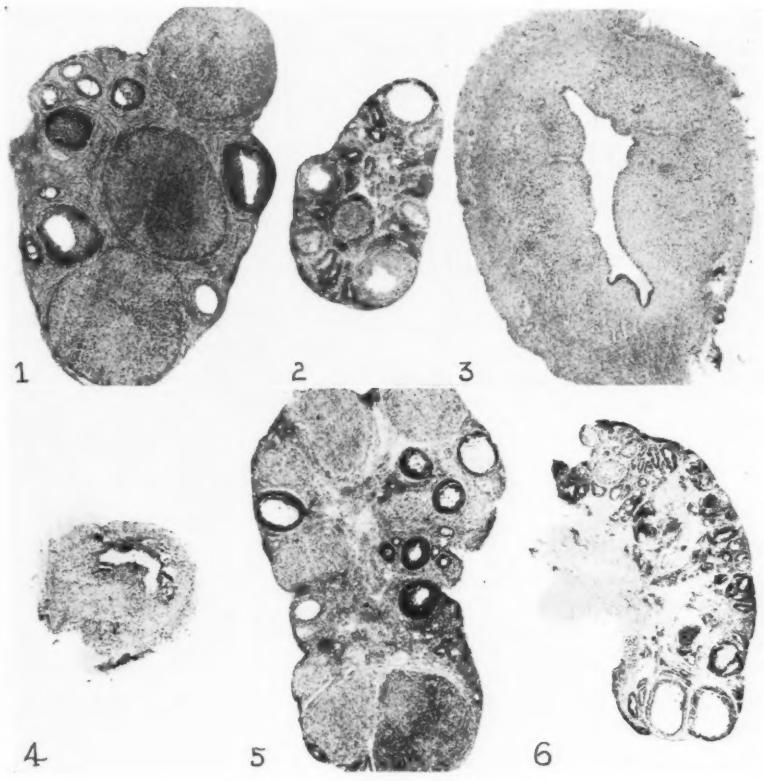


Fig. 1. Ovary of control rat (×18). Normal development of follicles and corpora lutea. Fig. 2. Ovary of treated rat (×18). Received testosterone propionate 0.5 gm. three times weekly for 102 days from infancy to maturity. Inhibition of ovarian growth, arrest of follicular development and absence of corpora lutea. Fig. 3. Uterine horn of control rat (×22). Normal state of horn. Fig. 4. Uterine horn of treated rat (×22). Received testosterone propionate 0.5 mg. three times weekly for 102 days from infancy to maturity. Marked atrophy of mucosa and myometrium. Fig. 5. Ovary of control rat (×18). Normal development of follicles and corpora lutea. Fig. 6. Ovary of treated rat (×18). Received testosterone propionate 0.5 mg. three times weekly for 62 days. Atrophy, degenerative changes in follicles and absence of corpora lutea.

The ovaries of the 15 animals which were killed the day following the last injection of testosterone propionate were atrophic and were only 33 per cent of the weight of control ovaries (table 2). Microscopically they showed degenerative changes in the follicles and an absence of corpora lutea (fig. 5 and 6). The uterine horns of the treated animals averaged 75 per cent of the weight of those of controls (table 2). Microscopic study showed atrophy of both mucosa and myometrium (fig. 8 and 9). There was definite inhibition

of estrus in all of the treated animals. Four of the 20 had no estrous cycles after the beginning of treatment. The remaining 16 animals had 1 to 3 estrous cycles during the early part of the experiment. Sections of the vagina

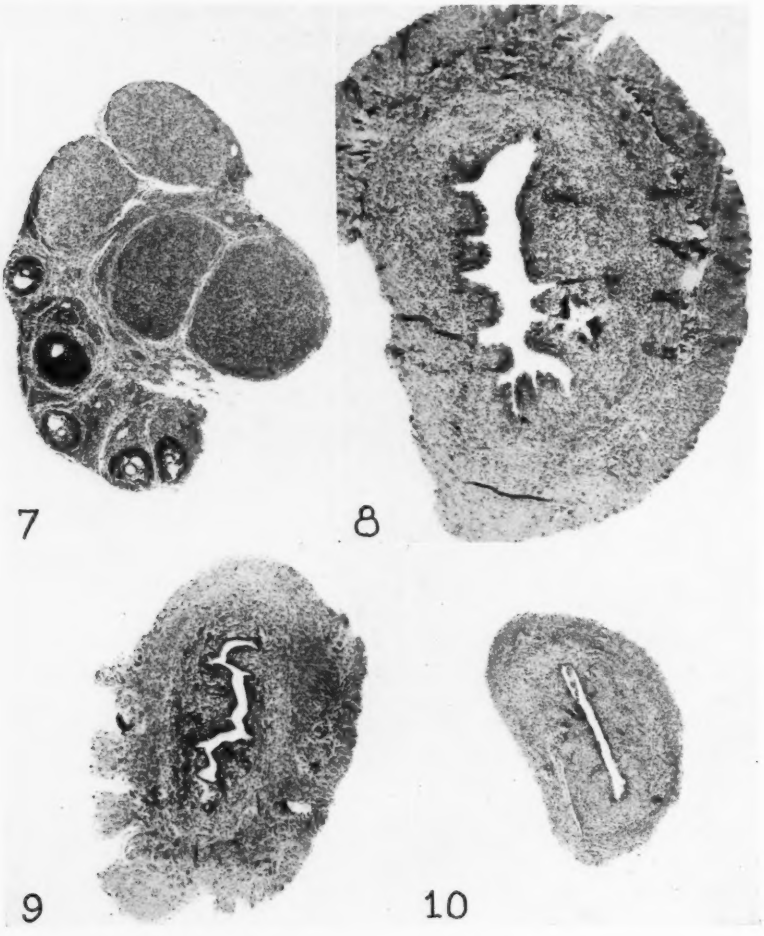


Fig. 7. OVARY OF TREATED RAT AFTER REST PERIOD (×25). Received testosterone propionate 0.5 mg. three times weekly for 62 days and sacrificed 29 days after the last injection. Recovery evident from follicular growth and presence of corpora lutea. Fig. 8. UTERINE HORN OF CONTROL RAT (×22). Normal state of horn. Fig. 9. UTERINE HORN OF TREATED RAT (×22). Received testosterone propionate 0.5 mg. three times weekly for 62 days. Atrophy of mucosa and myometrium. Fig. 10. UTERINE HORN OF TREATED RAT AFTER REST PERIOD (×22). Received testosterone propionate 0.5 mg. three times weekly for 62 days and sacrificed 29 days after the last injection. Note that the uterus had undergone further atrophy.

of the treated animals showed partial mucification. While the average weights of the pituitary glands of the treated and control animals differed appreciably (table 2), the deviations of the 2 sets of values from their means makes the

difference of doubtful significance. The adrenal glands of the treated animals were, however, appreciably smaller, only 69 per cent of the weight of the control adrenals (table 2). The thymus glands of the treated animals were grossly decreased in size, a finding also reported by Korenchevsky (1) with shorter treatment.

TABLE 2. EFFECT OF PROLONGED TESTOSTERONE PROPIONATE ADMINISTRATION ON THE ADULT RAT

	Average Weight of Organs		
	Control Rats (6)	Treated Rats (15)	Treated Rats (5) after 29-day Rest Period
Body Weight at Autopsy (gm.)	175	181	169
Ovaries (mg.)	61	20	34
Uterine Horns (mg.)	396	297	220
Adrenals (mg.)	42	29	
Pituitary (mg.)	9.4	7.7	

A study of the 5 animals which were killed 29 days after cessation of injections demonstrated in definite fashion that the deleterious effects of prolonged testosterone propionate administration described above are only temporary. The ovaries of these animals showed a 70 per cent weight recovery (table 2) and complete restitution of function as shown by follicular growth, ovulation and luteinization (fig. 7). The uterine horns, however, had undergone further atrophy (table 2) despite restitution of ovarian function. A possible explanation for this apparently anomalous phenomenon is given later. The withdrawal of treatment was followed by a recurrence of estrous cycles within 4 to 19 days (average 12).

DISCUSSION

The most pronounced effect, both anatomic and functional, resulting from the prolonged administration of testosterone propionate to the adult and immature rat is noted in the generative organs, as seen in the temporary but very marked atrophy of the ovaries and suppression of estrus. Atrophy of the ovaries with prolonged testosterone propionate administration (114 days) and their ability to recover after withdrawal of treatment was also observed in a rhesus monkey by Zuckerman (7). Menstruation, which was suppressed during the entire period of treatment, reappeared on the eighth day after withdrawal of testosterone. At this time the ovaries showed histologic evidence of follicular regeneration. That the response of the human ovary to the administration of testosterone propionate is similar to that of the experimental animal may be inferred from the temporary suppression of menstruation and arrest of endometrial growth which followed its use in adequate dosage (8, 9).

In the light of our present knowledge of the pituitary control of ovarian structure and function, it is reasonable to assume that pituitary inhibition resulting from the prolonged administration of testosterone is primarily responsible for the ovarian atrophy and consequent utero-vaginal effects herein reported. The marked arrest of pituitary growth in the treated im-

mature animals strongly suggests a concomitant inhibition of gonadotropic function responsible for the ovarian atrophy. The failure of the adult pituitary to decrease in weight significantly is not inconsistent with the assumption of inhibition of gonadotropic function, since it is well known that the adult pituitary does not readily respond to either stimulation or depression by gross change in weight. The decrease in size of the adrenals in the immature and adult rat alike supports this view, since it is well known that the anterior pituitary lobe controls the size of the adrenal cortex. The hypothesis that testosterone acts primarily through inhibition of the gonadotropic function of the anterior pituitary lobe and that the gonadal atrophy is secondary and sequential thereto is further supported by the observation that the substance prevents the occurrence of anterior lobe castration changes in the rat (10) and decreases the gonadotropic potency of its pituitary even after relatively short treatment (11). Similarly, in castrated men and women, the gonadotropic hormone excretion is decreased following large doses of testosterone propionate (12, 13).

The ovarian atrophy produced by prolonged treatment with testosterone is in contrast to the ovarian stimulation following relatively short treatment (1, 2, 3, 4) and stresses the importance of the time element in the administration of the substance. The length of treatment is also the determining factor in the effect of estrogen on the ovaries as described by Mazer et al. (14) and by Ellison and Burch (15).

The seemingly paradoxical finding by Hamilton and Wolfe (2, 11) of coexisting pituitary inhibition and ovarian stimulation by relatively short treatment of the rat with testosterone propionate may be explained either on the basis of a temporary release of accumulated gonadotropic hormone from the pituitary gland resulting in temporary ovarian stimulation, as suggested by the authors, or by the remote possibility of a direct stimulative effect on the ovary by testosterone. The latter possibility is suggested by the observations of Zwarenstein and Shapiro (16) that testosterone propionate causes ovarian stimulation even in the hypophysectomized amphibian.

Testosterone, like the estrogens, seems to have a direct stimulative effect on the uterus of the intact animal which is, however, counterbalanced by the failing ovarian function incident to its prolonged administration. This is suggested by the relationship between the uterine and ovarian weights in the treated immature rats (table 1). After a treatment period of 102 days, the ovaries of the treated animals had an average weight of only 10 mg., comparable to that of 22-day-old rats. With ovaries of this weight, uterine horns usually weigh about 25 mg. In this experiment, however, the uterine horns of the treated animals had an average weight of 178 mg. which is 7 times as great as one would expect in animals with ovaries weighing 10 mg. In other words, the inhibition of uterine growth did not parallel the inhibition of ovarian growth. More direct evidence of the uterine growth-promoting effect of testosterone is furnished by the observations of Korenchevsky et al. (1, 6) and of the authors (17) that uterine growth follows its administration to the castrated rat. It seems reasonable to conclude that the size of the

uterine horns in our experiments is the resultant of two opposing actions; first, the direct stimulation to growth by the testosterone and second, the decreasing estrogenic action on the uterus incident to the progressive ovarian failure during the administration of the testosterone. The application of this concept provides a reasonable explanation for the further uterine atrophy which occurred in the 5 adult rats after withdrawal of treatment (table 2). If on the withdrawal of testosterone propionate, the direct growth stimulating effect on the uterus ceases before it completely reacts to the increasing ovarian function, the organ must then at that stage be smaller than during the testosterone propionate action.

The mucifying effect of testosterone on the vagina associated with the ovarian stimulation reported by Korenchevsky and his associates (1) might at first be presumed to be due to the presence of corpora lutea. The vaginal mucification noted in our experiments where marked ovarian atrophy was present suggests another mechanism. This is to be found in the observations of Korenchevsky (6) and the authors (17) that testosterone produces mucification even in the vagina of the ovariectomized rat.

SUMMARY

In the immature rat, prolonged administration of testosterone propionate causes a decrease in the weight of the pituitary, adrenals, ovaries and uterine horns and inhibition of estrus with mucification of the vaginal epithelium. The degree of uterine atrophy is not in proportion to the ovarian atrophy, presumably because the suppression of estrogen production is partly counterbalanced by the direct stimulative effect of testosterone on the uterus. The adult rat reacts similarly with the exception that the pituitary gland shows no significant decrease in weight. The deleterious effect of prolonged testosterone treatment on the ovaries is temporary. Restitution of structure and function occurs within 29 days.

Evidence is drawn from the literature and the experiments described that the duration of treatment is the determining factor in the effect of testosterone on the ovaries; short treatment produces stimulation and prolonged treatment depression. It is considered that the ovarian atrophy herein described is secondary to pituitary inhibition.

REFERENCES

1. Korenchevsky, V., M. Dennison and K. Hall: *Biochem. J.* 31: 780. 1927.
2. Wolfe, J. M., and J. B. Hamilton: *Proc. Soc. Exper. Biol. & Med.* 37: 189. 1937.
3. McKeown, T., and S. Zuckerman: *Proc. Roy. Soc., London, s. B* 124: 362. 1937.
4. Salmon, U. J.: *Proc. Soc. Exper. Biol. & Med.* 38: 352. 1938.
5. McEuen, C. S., H. Selye and J. B. Collip: *Proc. Soc. Exper. Biol. & Med.*, 36: 390. 1937.
6. Korenchevsky, V., and K. Hall: *J. Path. & Bact.* 45: 681. 1937.
7. Zuckerman, S.: *Lancet* 2: 676. 1937.
8. Loeser, A. A.: *Lancet* 1: 373. 1938.
9. Foss, G. L.: *Lancet* 1: 992. 1938.
10. Wolfe, J. M., and J. B. Hamilton: *Endocrinology* 21: 603. 1937.
11. Hamilton, J. B., and J. M. Wolfe: *Endocrinology* 22: 361. 1938.
12. Frank, R. T., and U. J. Salmon: *Proc. Soc. Exper. Biol. & Med.* 34: 804. 1936.
13. Salmon, U. J.: *Proc. Soc. Exper. Biol. & Med.* 37: 488. 1937.
14. Mazer, C., S. L. Israel and B. Alpers: *Endocrinology* 20: 753. 1936.
15. Ellison, E. T., and J. C. Burch: *Endocrinology* 20: 746. 1936.
16. Zwarenstein, H., and H. A. Shapiro: Quoted by McKeown and Zuckerman (3).
17. Unpublished observations.

HUMAN SERUM RESPONSE TO GONADOTROPIC HORMONE (PREGNANCY URINE EXTRACT)

WILLIAM SAPHIR, KATHARINE M. HOWELL AND RALPH H. KUNSTADTER

From the Departments of Endocrinology, Serology, and the Pediatric Endocrine Clinic, Michael Reese Hospital

CHICAGO, ILLINOIS

THE TREATMENT of a series of cryptorchid children receiving large subcutaneous doses of gonadotropic hormone (pregnancy urine extract) offered us the opportunity of studying their blood for immunologic and biologic response to the hormone. Observations on the urinary excretions of the hormone in these same cases were previously reported by Hess, Kunstadter, and Saphir (1).

In this study we were concerned with the problem of serum response in patients receiving injections of the gonadotropic hormone. The importance of such investigation is apparent, particularly in view of the wide-spread use of hormones in present-day practice. If there is evidence of formation of an anti-hormone directed against the action of the hormone injected, the further administration of such a hormone would apparently be useless.

There is a great deal of experimental evidence in favor of such an assumption. In regard to the gonadotropic hormone, it has been established that with protracted administration the effect upon the ovaries gradually diminishes until finally there is no effect upon the ovaries at all. This is considered by some investigators to be due to the formation of anti-hormones. Collip's (2) fundamental studies indicate that the sera of animals injected with gonadotropic hormone develop anti-hormones which in turn exert a 'protective' antagonistic influence upon the action of the gonadotropin.

Endocrinologic Study

Sera of 12 patients, 9 cryptorchid children (bilateral and unilateral) and 3 adults, who had received various amounts of the hormone, were used in this study (table 1).

In order to demonstrate, if possible, the presence of anti-hormone in the sera of our patients, the following experiments were performed. Inasmuch as these experiments uniformly gave negative results they will not be reported here in great detail.

a), One cc. of the serum of the patient, diluted 1:10, was injected daily into mature, female white mice over a period of from 4 days to 5 weeks. Daily vaginal smears taken during this time did not show any disturbance in the normal estrous cycle. The animals were killed and the ovaries examined histologically. Microscopic

examination of the ovaries revealed no deviation from the histological normal appearance.

b), Three cc. of the patient's serum, diluted 1:10, was injected in divided doses into infantile, female white mice. The animals were killed and the ovaries examined histologically. Microscopic examination of the ovaries revealed no deviation from the normal histological appearance of immature ovaries.

c), Infantile female rats were injected with pregnancy urine extract (antuitrin-S, Parke, Davis) until a marked gonadotropic response was obtained. The animals, were then injected with our serum in increasing doses. No inhibition of estrus could be noted during injections. The animals were killed after the injections were stopped and the ovaries examined histologically. Microscopic examinations revealed no inhibition of the normal ovarian response to antuitrin-S. All the ovaries showed marked ovarian stimulation, such as folliculation, hemorrhagic follicles, and corpora lutea.

Immunologic Study

The in vitro immunologic response to injection of hormones has been studied extensively by precipitin and complement-fixation tests. In the pres-

TABLE 1

Case	R.U. of P.U. ext. patient had received	Disturbed estrous cycle in animals	Complement fixation test with unheated serum	Complement fixation with heated serum
1	1400	no	4 plus	neg.
2	900	no	4 plus	neg.
3	1600	no	4 plus	neg.
4	1200	no	4 plus	neg.
5	1200	no	4 plus	neg.
6	1500	no	4 plus	neg.
7	1000	no	4 plus	neg.
8	1400	no	4 plus	neg.
9	1600	no	4 plus	neg.
10	2800	no	4 plus	neg.
11	1900	no	4 plus	neg.
12	800	no	4 plus	neg.

ent studies, the complement-fixation reaction was chosen because it has been found to be the more delicate method. The complement-fixation reaction was performed according to the original Wassermann technic in 0.1 amounts. performed according to the original Wassermann technic in 0.1 cc. amounts. Two quantitative tests were made upon each serum: in one, the antigen, antuitrin-S, Parke, Davis (a highly purified extract of human pregnancy urine), was the variable factor, and in the other, the serum.

Titration of the Antigen. Different samples of antuitrin-S have practically the same antigenic titration. One-tenth to two-tenths cc. of the undiluted antigen was usually anti-complementary. Higher amounts were hemolytic. The antigenic unit with sera that reacted positively was usually 0.01 cc. of the undiluted antigen. Antigens diluted 1:10 and 1:100 with physiological saline solution did not produce satisfactory results. The antigen when stored in the ice box was stable for at least 2 months. Antigen boiled for 3 minutes or heated 56° C. for 30 minutes lost its antigenic power.

Serum. Sera from 12 persons injected with varied amounts of antuitrin-S

and control sera (negative and positive Wassermann sera) were tested by the complement-fixation procedure, using antuitrin-S as the antigen.

Results. The sera from the patients treated with antuitrin-S resulted in positive fixation when the sera were not inactivated. The control sera, as might be expected, gave an occasional weakly positive fixation. When the sera from the patients and the control sera were inactivated at 56° C. for 20 minutes no positive fixation could be obtained. Because of this result, the properties of the patients' sera were further studied. The sera were heated for different periods at 56° C.; the periods were 3, 5, 8, 10, and 15 minutes. Heating for 3 minutes at 56° C. reduced a strongly positive to a weakly positive reaction (1 or 2 plus); for 8 minutes at 56° C. to a complete negative. Adding guinea-pig complement to the inactivated sera failed to restore the ability to bind complement in the presence of antuitrin-S antigen. Therefore, complement is not the essential factor in the serum for the reaction. Absorbing native anti-sheep amboceptor from the sera tested did not modify the results. The binding power of the sera was not affected by exposure to ice or freezing with carbon dioxide snow. Bacterial contamination of the sera with staphylococcus, diphtheroids, or other bacteria did not affect the complement fixation reaction unless the contamination had caused the sera to become anti-complementary. Storage of sterile sera in the ice box for several months did not materially modify the complement fixation reactions of the sera.

Control Antigens. A Wassermann antigen, prepared according to Kolmer's method, and the antuitrin-S preparation, boiled for 3 minutes were used as control antigens in the complement-fixation tests. The inactivated sera from the patients and from normal controls gave negative complement-fixation results with these antigens. Two four-plus Wassermann sera were positive with the Kolmer antigen, but negative with the antuitrin-S and antuitrin-S heated antigen. These complement-fixation tests indicated that the sera from patients receiving gonadotropic hormone did not fix complement with non-specific antigen, and that sera from patients, even those with four-plus Wassermanns, who had not received this hormone, gave only an occasional weakly-positive fixation with the antuitrin-S antigen. The real problem was still unsettled, that is whether the sera from the patients treated with antuitrin-S contained a true anti-hormone or merely a non-specific urine-protein antibody induced by the protein in the concentrated urine preparation. Through the courtesy of Dr. Oliver Kamm, Parke, Davis & Company prepared the following product for use as control antigen in our study. A normal urine extract was prepared, each cc. representing 75 cc. of urine, comparable in its preparation to antuitrin-S. In antuitrin-S preparation, 50 to 100 cc. of pregnancy urine, according to the amount of hormone present, are necessary to produce 1 cc. of the product containing 100 R.U. of the hormone. The normal female urine extract was, for practical purposes, inert since it had a potency of only 0.15 R.U. per cc. Unfortunately only a few sera

were available when this normal urine extract was received, but these sera (active) gave positive complement fixation tests when this concentrated normal urine extract was used as an antigen. These results correspond to those reported by Sulman (3), and indicate that the appearance of the immune bodies is probably not in response to the gonadotropic hormone content of the urine extracts. In this connection we have also used sera from pregnant women, which are known to contain large amounts of gonadotropic hormone, in our complement fixation tests. The results were usually negative confirming the view that the presence of gonadotropic hormone per se in the blood does not result in immune body formation.

COMMENT

As the result of the above experiments it appeared that an anti-gonadotropic factor could not be demonstrated in the serum of humans receiving large doses of gonadotropic hormone. It would be reasonable to assume that if the serum contained an antigonadotropic factor, its injection into female white mice over a period up to 5 weeks would result in diminution or cessation of the normal estrous cycle. This, however, could never be noted. Our serum, therefore, apparently was free from anti-hormone.

Antuitrin-S injected into patients gave rise to some factor in their sera that fixed complement in the presence of the antigen antuitrin-S. We found this anti-substance to be thermolabile, losing its binding power for complement when heated for 8 minutes at 60° C. This thermolabile property has also been noted by Laroche (4), who found that of his 58 positive sera only 8 remained positive after heating. This differs from the findings of Zondek and Sulman, (5) who found that the anti-gonadotropic factor was not destroyed by heating to 70° C. for 1 hour, but was destroyed at 80° C. It is difficult to conceive of either a true antibody or a true anti-hormone so thermolabile. The question arises as to whether this factor might be an enzyme or a ferment, but even these are usually not so heat labile. Zondek and Sulman (6), who made an exhaustive study as to the origin of this anti-gonadotropic substance, concluded that antiprolan is neither an anti-hormone nor a ferment, but possibly a new kind of factor approaching very closely the immune bodies to which it is in some respect quite similar. The result of our own study would seem to support this point of view.

Ehrlich (7), Collip (2), and many others have considered the antagonistic factor to be an anti-hormone. From the present work it is apparently inadvisable to classify the antagonistic factor as an anti-hormone, first because anti-hormone was not demonstrated in the biologic tests, admittedly the more sensitive criteria, although they do not rule out the presence of anti-hormone, since it has been concluded by Bachman (8) and others that the in vitro and in vivo tests for the presence of anti-hormone do not necessarily run parallel; second, because the antagonistic factor in the serum of the patients treated with gonadotropic hormone fixed complement with extracts of

normal urine as well as with extracts of pregnant urine, when used as antigens.

SUMMARY

Sera of human beings receiving large doses of gonadotropic hormone (pregnancy urine extract) were examined for the presence of anti-hormones and for complement fixation antibodies.

Injection of the sera into females and immature female white mice over a prolonged period of time did not produce any noticeable changes upon the normal estrus cycle nor histologic changes in the ovaries. Injection of the sera into infantile female rats failed to inhibit the gonadotropic response produced by injection of antuitrin-S. Antuitrin-S injected into patients illicited a thermolabile factor in their sera that fixed complement in the presence of antuitrin-S antigen (pregnancy urine extract) and in the presence of an extract of normal urine used as antigen.

It is concluded that human beings do not respond with either true antibody or anti-hormone formation following the injection of gonadotropic hormone.

We are indebted to Dr. Samuel Soskin for cooperation and advice.

REFERENCES

1. Hess, J. H., R. H. Kunstadter and W. Saphir, *J.A.M.A.* 108: 352. 1937.
2. Collip, J. B.: *Ann. Int. Med.* 9: 150. 1935.
3. Sulman, F.: *J. Exper. Med.* 65: 1. 1937.
4. Laroche, G., R. Demanche, H. Simonnet and Lebeau: *Compt. rend. Soc. de biol.* 121: 659. 1936.
5. Zondek, B., and F. Sulman: *Proc. Soc. Exper. Biol. & Med.* 37: 193. 1937.
6. Zondek, B., and F. Sulman: *Proc. Soc. Exper. Biol. & Med.* 37: 343. 1937.
7. Ehrlich, H.: *Wien. klin. Wchnschr.* 47: 1323. 1934.
8. Bachman, C.: *Proc. Soc. Exper. Biol. & Med.* 32: 851. 1935.

FURTHER STUDIES OF MALE SEX-STIMULATING, FEMALE SEX-REPRESSIVE FRACTIONS OF THE ADRENAL GLANDS OF COWS, STEERS AND BULLS[1]

F. M. POTTENGER, JR. AND D. G. SIMONSEN
MONROVIA, CALIFORNIA

IN A PREVIOUS PAPER, we (1) described a female sex-repressive, male stimulative factor prepared from adrenal glands. This work has been extended to a study of the differences of this fraction as obtained from cow, bull and steer adrenal glands.

The method of preparation described in the previous paper was used except for one minor change. The benzene-insoluble material (after agitation with acetic acid and sodium acetate) is rather difficult to filter. We found that the addition of a small amount of Celite (diatomaceous earth) aids greatly in the filtration. In some cases, however, even after addition of Celite, the solution may filter slowly. If this occurs the mixture should be frozen solid (−20° C. will suffice) for from 24 to 48 hours. The freezing breaks the colloid mass and upon thawing the aqueous solution separates from the fatty material and can be readily filtered.

The female sex-repressive, male stimulative factor (hereafter called the *S-S* fraction) was prepared separately from bull, cow and steer adrenals. The concentrations of the various preparations were as follows: bull gland preparation 190, 1 cc. equivalent to 56 gm. of whole gland; 235, 40 gm. of whole gland (bull adrenals are very difficult to obtain and hence the concentration had to be reduced in preparation 235). Cow gland preparations 176 and 234, 1 cc. equivalent to 56 gm. of whole gland. Steer gland preparations 120 and 159–60, 1 cc. equivalent to 66 gm. of whole gland.

The male and female test rats were given injections (subcutaneous) of 0.5 cc. of these various fractions daily for 30 days. The control rats were litter mates (litter mates are designated by letters in the tables) of the test animals and were untreated. Both test and control animals were weighed at 10-day intervals. The rats were from 2.5 to 6 months of age. Daily vaginal smears were made of both the test and control female rats. The smears were graded on a scale of 0 to 4 as suggested by Frank (2). The scale is as follows: 0, leucocytes, mucus, a few nucleated epithelial cells; 1, predominance of leucocytes, increase in nucleated epithelial cells; 2, presence of numerous leucocytes, predominance of nucleated epithelial cells; 3, no leucocytes, nucleated epithelial cells; 4, epithelial non-nucleated scales exclusively. At the end of the 30-day period, the animals were sacrificed. The uteri and ovaries of the female rats and the testes of the male rats were removed and weighed.

RESULTS

Tables 1, 2 and 3 show the weights of the animals and of the uteri, ovaries and testes both of the control rats and of those treated with the *S-S* fraction of steer, cow and bull adrenals respectively.

[1] Presented before the 22nd Annual Scientific Meeting, of the Association for the Study of Internal Secretions, San Francisco, California, June 14, 1938.

Steer Glands. Although marked variations were observed among the individual rats, the uteri and ovaries of the test animals per group were smaller than those of the control animals. In the test animals the weight of the uteri and ovaries ranged from 0.136 to 0.225 gm. and in the controls from 0.170 to 0.224 gm. Calculated in

TABLE 1. S-S FRACTION FROM STEER ADRENALS[1]

	Rat No.	Body Wt.	Wt. of Uterus and Ovaries	Wt. of Uterus and Ovaries / Body Wt. %	Average %
		Grams			
		A. Female Rats			
Prepn. 159–60	S-54	109	0.144	0.132	
Test animal	S-80	114	0.199	0.175	0.153
Control animal	S-50	102	0.190	0.186	
	S- 7	118	0.170	0.144	0.165
Prep. 159–60					
Test animal	M-1	88	0.136	0.155	
	L-2	93	0.225	0.242	0.196
Control animal	M-20	90	0.224	0.249	
	L-13	110	0.198	0.180	0.214
			Wt. of Testes	Wt. of Testes / Body Weight %	
		Grams			
		B. Male Rats			
Prepn. 120					
Test animal	F-9	87	1.30	1.49	
	F-6	95	1.60	1.68	
	H-50	81	1.10	1.36	
	G-40	95	1.70	1.77	1.57
Control animal	F-5	110	1.70	1.54	
	F-4	94	0.70	0.74	
	H-55	96	1.20	1.25	
	G-44	89	1.60	1.79	1.33
Prepn. 159–60					
Test animal	R-84	118	1.63	1.38	
	R-70	129	1.74	1.35	1.37
Control animal	R-59	107	1.55	1.45	
	R-90	131	1.42	1.08	1.27
Prepn. 159–60					
Test animal	M-11	104	1.58	1.52	
	M-22	101	1.56	1.54	1.53
Control animal	M-10	101	1.49	1.48	
	M-21	95	1.38	1.45	1.47

[1] The figures in this table were compiled from a paper previously published by the authors. (1)

terms of per cent of body weight, the uteri and ovaries of the test animals average 10 per cent less than those of the controls.

Vaginal smears were not made of this group. However, in another experiment (3) vaginal smears were made for 60 days on a group of female rats used for tests with the S-S fraction prepared from steer glands. The controls showed a normal cycle, 5 to 7 days intervening between the four reactions. In the test animals there was usually

a delay in the first full reaction which on an average was 10 days. Then a marked stimulation occurred, the scale going from 0 to 4, then back to 2, and up again to 4, the rats never reaching a typical anestrous stage. This lasted for about 15 days. From

TABLE 2. S-S FRACTION FROM COW ADRENALS

	Rat No.	Body Wt.	Wt. of Uterus and Ovaries	Wt. of Uterus and Ovaries / Body Weight %	Average %
		Grams			
		A. Female Rats			
Prepn. 176					
Test animal	1V-1	96	0.097	0.101	
	1W-5	86	0.078	0.091	
	1Y-13	80	0.046	0.057	
	1Z-15	87	0.068	0.078	0.082
Control animal	1V-2	82	0.138	0.169	
	1W-6	105	0.184	0.175	
	1Y-14	92	0.181	0.197	
	1Z-16	92	0.064	0.069	0.152
Prepn. 234					
Test animal	2P-26	137	0.254	0.185	
	2Q-33	164	0.325	0.198	
	2Q-35	140	0.388	0.277	
	2R-42	66	0.104	0.158	0.205
Control animal	2P-30	153	0.313	0.204	
	2Q-34	152	0.323	0.212	
	2Q-36	131	0.423	0.323	
	2R-43	127	0.279	0.220	0.240
			Wt. of Testes	Wt. of Testes / Body Wt. %	
		Grams			
		B. Male Rats			
Prepn. 176					
Test animal	1W-3	119	1.45	1.22	
	1X-9	106	1.41	1.33	
	1Y-11	117	1.46	1.25	
	2A-20	130	1.63	1.25	1.26
Control animal	1W-4	125	1.58	1.26	
	1X-10	124	1.36	1.10	
	1Y-12	127	1.48	1.17	
	2A-21	114	1.28	1.12	1.16
Prepn. 234					
Test animal	2P-22	183	2.38	1.30	
	2P-24	149	2.42	1.62	
	2Q-31	166	2.26	1.36	
	2R-40	161	2.27	1.41	1.42
Control animal	2P-23	177	2.41	1.36	
	2P-25	187	2.59	1.39	
	2Q-32	159	2.23	1.40	
	2R-41	190	2.39	1.26	1.35

then on the reaction diminished in intensity and the peaks of the cycles lengthened. In three-fourths of the rats the estrous cycle disappeared in from 30 to 35 days and at the end of 60 days the vaginal smear was graded 0. Although variations were

noted in the individual rats the above is a general picture for the group studies.

In the male rats, there was an increase in the weights of the testes (calculated as per cent of body weight) of the test animals as compared to those of the controls.

TABLE 3. S-S FRACTION FROM BULL ADRENALS

	Rat No.	Body Wt.	Wt. of Uterus and Ovaries	Wt. of Uterus and Ovaries / Body Wt. %	Average %
		Grams			
		A. Female Rats			
Prepn. 190					
Test animal	1R-11	107	0.280	0.262	
	1S-15	98	0.250	0.255	
	1T-20	94	0.121	0.129	
	1U-22	80	0.119	0.149	0.199
Control animal	1R-12	108	0.404	0.374	
	1S-16	91	0.143	0.157	
	1T-21	96	0.107	0.112	
	1U-23	79	0.141	0.178	0.205
Prepn. 235					
Test animal	2M-1	142	0.310	0.218	
	2N-11	132	0.339	0.257	
	2O-13	134	0.316	0.236	
	2O-15	152	0.377	0.248	0.240
Control animal	2M-2	150	0.403	0.269	
	2N-12	159	0.389	0.244	
	2O-14	141	0.350	0.248	
	2O-16	150	0.362	0.242	0.251
			Wt. of Testes	Wt. of Testes / Body Wt. %	
		Grams			
		B. Male Rats			
Prepn. 190					
Test animal	1P-1	—	—	—	
	1Q-3	114	1.29	1.13	
	1Q-5	114	1.25	1.10	
	1S-13	95	0.72	0.76	1.00
Control animal	1P-2	—	—	—	
	1Q-6	98	0.78	0.79	
	1Q-40	117	0.79	0.68	
	1S-14	68	0.46	0.67	0.71
Prepn. 235					
Test animal	2M-3	161	2.68	1.67	
	2N-5	195	2.49	1.28	
	2N-9	154	2.35	1.53	
	2O-20	135	2.01	1.49	1.49
Control animal	2M-4	186	2.71	1.46	
	2N-6	194	2.49	1.28	
	2N-10	187	2.63	1.41	
	2O-21	191	2.22	1.16	1.33

In the test animals the average weight of the testes per group ranged from 1.10 to 1.74 gm. and in the controls from 0.70 to 1.70 gm. On an average group basis the testes of the treated animals were from 5 to 15 per cent larger than those of the untreated controls.

The weights of both test and control animals (male and female) increased during the time of injections. Variations were noted but based on group averages the changes were unimportant.

Histological observations of the female rats showed that the control animals had a greater follicular development than the test animals and also that the controls had a more highly developed type of uterus. In the uteri of the test animals degeneration of underlying epithelial cells was found. In the males, the test animals revealed a more mature spermatogenesis and the tubules showed greater activity as compared to those of the controls.

Cow Glands. There was a much greater degree of atrophy noted in the uteri and ovaries of the group used for tests with the S-S fraction of cow adrenals than with that of steer adrenals. In the test animals the weights of the uteri and ovaries ranged from 0.046 to 0.388 gm., and in the controls from 0.064 to 0.423 gm. Calculated in terms of percentage of body weight the uteri and ovaries of the test animals were from 15 to 45 per cent smaller than those of the controls.

Vaginal smears were made daily for 30 days in this group. The picture was somewhat similar to that obtained from rats treated with the S-S fraction of steer glands except that there was no stimulation of the cycle. The estrous cycle gradually diminished in intensity; after 10 to 15 days of treatment most of the rats showed only a 2 reaction. The cycle gradually disappeared and at the end of 30 days the reaction was graded 0, having been at that point for the last 10 to 15 days. The controls showed a 5 to 7-day cycle.

The female rats treated with S-S fraction 176 showed an average loss in weight of 1 gm. per rat as compared to an average gain of 5.5 gm. per control rat. These rats were approximately 6 months of age at the beginning of the experiment. In those treated with 234, the test animals gained (average per rat) 30 gm. as compared to 46 gm. for the control rats. The rats in this group were 2.5 months of age at the beginning of the experiment.

The histological picture of the uteri and ovaries was similar to that obtained on rats treated with steer adrenals, except that the changes were much more marked.

In the male rats there was only a slight stimulation of growth of the testes of the animals treated with the S-S fraction of cow glands. In the test animals the weight of the testes ranged from 1.41 to 2.42 gm., and in the controls from 1.28 to 2.59 gm.

The test animals gained an average of 3 gm. per rat and the controls 13 gm. per rat during the course of treatment with S-S fraction 176. In those treated with 234 the test animals gained an average of 56 gm. while the controls gained 67 gm. during the 30 day treatment. The first group averaged 6 months of age and the second, 2.5 months at the beginning of the experiment.

No histological observations were made on the testes of the rats treated with cow gland extract.

Bull Glands. There was only a slight difference in the weights of the uteri and ovaries of the test and control animals of this group. Those of the test animals ranged from 0.119 to 0.377 gm. and in the controls from 0.107 to 0.403 gm.

There was no inhibition of the estrous cycle of the female rats treated with the S-S fraction of bull glands. Although some variations in the individual rats were observed, both test and control animals showed a 5 to 7-day cycle.

The test females treated with S-S fraction 190 showed an average gain in weight of 3 gm., the controls, 9 gm. Those treated with 235 an average gain of 41 gm., the controls 59 gm. The groups of rats were 6 and 2.5 months of age respectively.

No histological examinations were made of the organs of the female rats of this group.

The testes of male rats treated with the S-S fraction of bull adrenals showed the greatest increase in weight compared to those of steer and cow adrenals. The weights of the testes of the treated animals ranged from 0.72 to 2.68 gm. and in the controls from 0.46 to 2.71 gm. Based on terms of percentage of body weight the testes of the treated animals were from 12 to 29 per cent larger than those of the controls.

Of the group used for tests with S-S 190 the treated animals showed an average gain of 11 gm., the controls 7 gm.; 235, the treated animals 48 gm., the controls, 66 gm.

Histological observation of the testes of the treated male rats showed a much more mature spermatogenesis and the tubules showed greater activity than those of the controls.

GENERAL DISCUSSION

Table 4 gives a summary of the data in tables 1, 2 and 3. Briefly, the S-S fraction from cow adrenals produces a marked atrophy of the uteri and

TABLE 4. SUMMARY OF DATA

	Wt. of Uterus and Ovaries Body Weight Average per cent per group.	
	Test	Control
	A. Female Rats	
Bull	0.199	0.205
	0.240	0.251
Cow	0.082	0.152
	0.205	0.240
Steer	0.153	0.165
	0.196	0.214
	Weight of Testes	
	Body Weight Average per cent per group	
	Test	Control
	B. Male Rats	
Bull	1.00	0.71
	1.49	1.33
Cow	1.26	1.16
	1.42	1.35
Steer	1.57	1.33
	1.37	1.27
	1.53	1.47

ovaries of female rats, ranging from 15 to 45 per cent based on per cent of body weight and has little effect on the testes of male rats. That prepared from bull glands increases the weight of the testes of the treated animals from 12 to 29 per cent and has little or no effect on the uteri of female rats. That of steer glands is intermediate in effect, i.e., it causes some atrophy of the uteri and ovaries of female rats (about 10 per cent) and a slight stimulation (5 to 15 per cent) of the growth of the testes of male rats.

In the majority of the female rats, the S-S fraction of cow adrenals inhibits the estrous cycle in 30 days, steer glands in 60 days and bull adrenals have no apparent effect.

There was no evidence of masculinization of the female rats. Mammary development was normal and there was no hypertrophy of the clitoris. Testosterone propionate produces a masculinization of female rats as reported by Hamilton (4). Korenchevsky and collaborators (5, 6) have reported an hypertrophy of the uterus and ovaries of female rats treated with testosterone propionate. The male sex hormones testosterone, androsterone and adrenosterone are closely related chemically. Adrenosterone has been isolated from the adrenal by Reichstein (7). This compound, or one similar to it, may be responsible for the effects produced in the male rats, which have been reported here. It apparently is not the compound which causes atrophy of the female sex organs.

Histological observations of the female rats showed that the control animals had a greater follicular development than the test animals and also that the controls had a more highly developed type of uterus. In the uteri of the test animals degeneration of underlying epithelial cells was found. In the males, the test animals revealed a more mature spermatogenesis and the tubules showed greater activity as compared to those of the controls.

SUMMARY

A female sex-repressive, male stimulative fraction has been prepared from cow, bull and steer adrenals.

The uteri and ovaries of female rats treated with this fraction prepared from cow glands were from 1/6 to nearly 1/2 the size of those of untreated controls. This same fraction had only a slight stimulative effect on the testes of male rats. This fraction from bull adrenals produced a 12 to 29 per cent increase in the weight of the testes of treated male rats as compared to those of the untreated controls. It produced only a slight atrophy of the uteri of female rats. This same fraction prepared from steer glands has an intermediate effect. On female rats the atrophy of the uteri amounts to about 10 per cent and the stimulation of testicular growth ranges from 5 to 15 per cent.

The estrous cycle is gradually inhibited in female rats treated with this fraction. Treatment for 60 days with steer glands or 30 days with cow glands produces a negative vaginal smear while that of rats treated with bull adrenals remains normal.

Histological observation reveals a functional atrophy of the uteri and ovaries of female rats and an increase of spermatogenesis in the testes of male rats.

REFERENCES

1. Pottenger, F. M., Jr., and D. G. Simonsen: *Endocrinology* 22: 197. 1938.
2. Frank, R. T.: The Female Sex Hormone. Thomas, 1929.
3. Pottenger, F. M., Jr., and D. G. Simonsen: Unpublished data.
4. Hamilton, J. B.: *Anat. Rec.* 70: 433. 1938.
5. Korenchevsky, V., M. Dennison and K. Hall: *Biochem. J.* 31: 434. 1937.
6. Korenchevsky, V., and K. Hall: *J. Path. Bact.* 45: 681. 1937.
7. Reichstein, T.: *Helv. Chim. Acta* 19: 223. 1936.

A COMPARISON OF THE RESISTANCE OF MALE AND FEMALE RATS TO CORTIN

DWIGHT J. INGLE

From the Division of Biochemistry and the Division of Experimental Medicine, The Mayo Foundation

ROCHESTER, MINNESOTA

WHEN LARGE AMOUNTS of cortin are administered to normal male rats there occur atrophy of the adrenal cortices, atrophy of the thymus, cessation of body growth and loss of general body weight (1). In the present experiment male and female rats are compared in respect to their resistance to large amounts of cortin.

METHODS

All of the rats used in this experiment had an initial body weight of 180 gm. Thirty male rats and 22 female rats were killed at this body weight in order to obtain control data on the weights of the adrenals and of the thymus. Thirty male rats and 32 female rats were treated for 1 week with varying amounts of cortin, which was administered in the drinking water. The choice of this method of administering cortin was based on previous comparisons (1, 2) of the drinking water method with the daily injection method in respect to its effects on the cortico-adrenal tissue of normal rats. Ten male rats and 5 female rats were hypophysectomized by the usual ventral parapharyngeal approach. After operation these animals were given a 4 per cent solution of *d*-glucose to drink. All of the animals were fed a commercial diet which contains 0.24 per cent sodium and 1 per cent potassium. All of the experimental animals were killed for necropsy by exsanguination. The adrenal glands were weighed by a standardized procedure. The maximal error in relative values for the weights of pairs of adrenal glands probably does not exceed 2 mg.

RESULTS AND COMMENT

The data on body weights and thymus weights are summarized in table 1. The data on weights of the adrenal glands are presented in figure 1. It is evident from inspection of the data that female rats are much more resistant to the administration of cortin than are male rats having a similar body weight. It was necessary to administer larger amounts of cortin to the female rats than to the males in order to produce a loss in body weight, a loss in the weight of the thymus or a loss in the weight of the adrenal glands. Histologic examination of the adrenal glands showed that when atrophy occurred it was confined to the cortex.

Higgins, Kendall and I have observed that the crystalline compounds *A* and *B* occurring in cortico-adrenal substance which have the property of maintaining the life of adrenalectomized animals have also the property of

TABLE 1. EFFECTS OF CORTIN ON BODY WEIGHT AND WEIGHT OF THE THYMUS OF MALE AND FEMALE RATS: AVERAGES AND RANGE

No. of rats		Cortin daily, cc.	Body weight, gm.		Thymus weight, mg.	
Males	Females		Males	Females	Males	Females
30	22	0	180[1]	180[1]	400.3 280–510	329 205–448
10	10	2	202.9 184–220	185.4 176–194	301 175–433	230 100–344
10	10	5	186.6 173–204	189.9 175–203	79 40–117	281.8 223–356
10	5	10	151.7 142–162	194 184–202	53.7 40– 76	270.2 220–309
0	5	20		175 170–180		99 46–126
0	2	40		160 155		70 67
10	5	Hypophysectomy	143 138–151	147 125–156		

[1] This is the original weight of all animals used in this experiment.

causing a loss in body weight, atrophy of the adrenal cortices and atrophy of the thymus in male rats. It is assumed that the changes reported here are due to the presence of the compounds essential for life in the extracts used.

King (3) failed to find any changes from normal among female rats following the oral administration of large amounts of cortico-adrenal substance. However, the amounts of cortico-adrenal substance administered daily in his experiment were much smaller than the minimal amounts which produced changes from normal among the female rats in the present experiment.

Evidence has previously been presented (4) that the effect of massive amounts of cortin on the adrenal cortices of the male rat may be mediated by the adrenotropic activity of the pituitary and that the atrophy of the adrenal cortex which follows the administration of cortin and the similar atrophy which follows the removal of the anterior lobe of the pituitary may be due to the same cause, namely, a decrease in the amount of adrenotropic hormone in the body fluids. In the present experiment it was observed that the atrophy of the adrenal cortices in either male or female rats may be just as marked after the administration of cortin for one week as after a similar period of hypophysectomy.

The greater resistance of the female rat to cortin may or may not be causally associated with the larger amount of cortical tissue in the adrenal glands of the female. Although the amount of cortical tissue in male and female rats is approximately equal at birth the difference develops soon after birth and persists throughout life. The difference develops and persists even when the animals of each sex are castrated at birth. There is no satisfactory explanation either for the sex difference in the amount of cortical tissue or for the sex difference among normal rats in resistance to large amounts of the cortical hormone.

In addition to the anatomic changes reported here there are probably other important cellular changes of which I am not aware which follow the administration of large amounts of cortin. My histologic studies on the tissues of these animals have been confined to the adrenal glands.

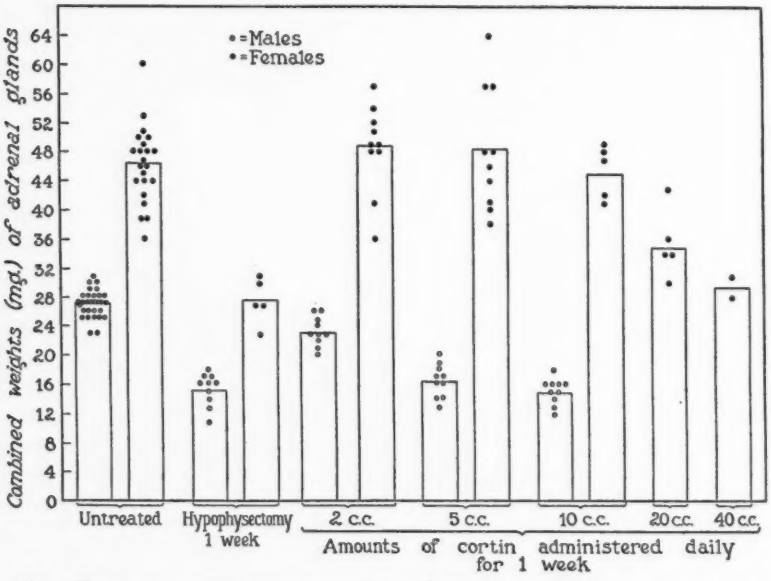


Fig. 1. The effect of cortin on the weight of the adrenal glands of male and female rats.

There have been numerous studies of the effects on normal animals of the administration of cortin. The resistance of normal animals to the effects of overdosage has not been generally recognized. Not only is it probable that the normal animal has the ability to excrete or destroy cortin without utilization but it also has the capacity to reduce the secretory activity of its own glands to a low level of activity.

SUMMARY

Male and female rats each having an initial body weight of 180 gm. were compared in respect to their resistance to the oral administration of large amounts of cortin for a period of 1 week. The female rat is more resistant than is the male to overdosage with cortin. It was necessary to administer larger amounts of cortin to females than to males in order to produce a loss in the weight of the adrenal glands or the thymus, or in general body weight. In each sex the maximal atrophy of the adrenal cortices which was induced by the administration of cortin was similar in extent to that which occurs during 1 week in the hypophysectomized animal.

REFERENCES

1. Ingle, D. J., G. M. Higgins and E. C. Kendall: *Anat. Rec.* 71: 363. 1938.
2. Ingle, D. J., and G. M. Higgins: *Am. J. M. Sc.* 196: 232. 1938.
3. King, J. L.: *Proc. Soc. Exper. Biol. & Med.* 35: 618. 1937.
4. Ingle, D. J.: *Am. J. Physiol.* (In press.)

THE REFRACTORY STATE PRODUCED BY ADRENAL EXTRACT[1]

FRANK A. HARTMAN, LENA A. LEWIS AND KENNETH P. McCONNELL

From the Department of Physiology, The Ohio State University
COLUMBUS, OHIO

IT HAS BEEN reported previously that single injections of large amounts of adrenal extract will cause marked changes in electrolyte excretion in the normal man (1) and the normal dog (2, 3). This effect is differential, the excretion of sodium and chloride being decreased and that of potassium and ammonia increased. The total base excreted remains essentially unchanged because the differential action is compensatory.

In a recent report (3) from this laboratory it was shown that repeated intravenous injections of adrenal extract eventually produce a condition in which there is no response. However, Harrop and Thorn (2) failed to observe the development of a refractory state following repeated subcutaneous injections of adrenal extract.

In the present investigation we sought to determine the conditions necessary for the development of the refractory state. We have found that it is developed by intravenous or intraperitoneal injection but not by subcutaneous injection; moreover, we have observed that it is species specific.

METHODS

The animals used in these experiments were all normal and consisted of adult dogs, 3 males (7 to 17 kg.) and 5 females (7 to 14 kg.); 3 adult female cats (2 to 3 kg.) and 1 young female pig (at 6 weeks of age, when first put on the diet, her weight was 20 kg. and after 3 months, at the end of the study, her weight was 70 kg.).

The procedure previously outlined for dogs (3) was carried out in detail for both dogs and cats with the exception of the diet in the case of the latter. This consisted of 50 gm. of canned salmon and 50 gm. of beef heart, daily. At the beginning of a 6-hour experimental period 25 cc. of water was given by stomach tube. In the animals tested over a 24-hour period the procedure was that described by Harrop and Thorn (2) except that in some instances injections were made intraperitoneally.

In the study of the pig, the diet consisted of 200 gm. of oats, 200 gm. of corn and 4 liters of milk plus 2 gm. of sodium chloride twice daily, at 3 P.M. and again at 11 P.M. (9 hours before the beginning of a test period), and 2 liters of milk plus 2 gm. of sodium chloride at 7 A.M., 1 hour before the beginning of the test period. The latter feeding was necessary as little or no sodium chloride was excreted during the experimental period if the last feeding was at 11 P.M. The experiments were all extended over a 6-hour period. The samples were collected by catheterization at the beginning and at the end of the period.

[1] Aided by a grant from the Rockefeller Foundation.

All extracts were prepared from whole adrenal glands. The Wilson Laboratories contributed one type of commercial extract while The Upjohn Company contributed another type. All other extracts were prepared in our own laboratories by the method of Cartland and Kuizenga (4). Beef, pig, sheep and horse adrenals were used. All extracts including the commercial preparations were assayed in our laboratory on cats (5). The maximum amount of extract injected at any one time was 10 cc. Usually it was 5 cc. or less.

The extracts never contained more than 1:75,000 epinephrin according to the colorimetric method of Folin, Cannon and Denis (6) which gives high values. Injections of the same amount of epinephrin were made in control experiments.

A comparison was made of the effect of repeated injection by different pathways, subcutaneous, intraperitoneal and intravenous. Intravenous injections were made into the radial vein of the dog, the jugular or saphenous vein in the cat and the marginal ear vein in the pig.

A study was made of the species specificity of the refractory state by injecting extract from adrenals of a second species after refractoriness had developed to a first species.

The possibility that on intravenous injection a combination of the hormone with the plasma proteins to form a haptene antigen occurs, suggested experiments in which 7.5 cc. of cortical extract (60 C.U.) was mixed with 5 cc. of sterile pig serum and incubated at 37° C. for 1 hour before subcutaneous injection. The experimental procedure was the one followed in the other experiments involving subcutaneous injections.

Frequent tests of the urine for albumin were made and microscopic examinations were performed. The analytical methods used were those outlined in the earlier work (3).

RESULTS

Subcutaneous Injection of Adrenal Extract. Following repeated subcutaneous injections a refractory state failed to develop. This was in agreement with the findings of Harrop and Thorn (2). Two male dogs injected 7 and 8 times respectively over a period of 3 to 4 weeks, showed no decrease in response. Figure 1 shows the results obtained for one of these animals. A female dog which received 25 subcutaneous injections over a period of 6 months gave similar results.

The one dog which received adrenal extract plus pig serum showed no decrease in response after 8 subcutaneous injections extending over one month. The average excretion in 24 hours without injection (9 determinations) was: Na, 54.00 m.eq. (range 51.45 to 59.5 m.eq.); K, 26.6 m.eq. (19.2 to 28.8 m.eq.); NH_3, 31.4 m.eq. (21.5 to 34.0 m.eq.). In the first experiment a preparation containing 60 C.U. of cortin plus 5 cc. of pig serum was injected at 8 A.M. and 5 P.M. The excretion for the 24-hour period was: Na, 41.5 m.eq.; K, 33.8 m.eq. and NH_3, 38.7 m.eq. The eighth injection of a similar dose of the same extract gave similar results: Na, 38.2 m.eq.; K, 36.2 m.eq. and NH_3, 35.8 m.eq.

Intraperitoneal Injection of Adrenal Extract. Our use of cats in this study proved rather unsatisfactory because it was difficult to obtain consistent control values. However, the 3 animals tested seemed to develop the refractory state from intraperitoneal injection. Of these, one gave consistent con-

trols so that the refractory state was unquestionable. It required 8 injections (32 c.u. each) over a period of 7 weeks to make the animal completely refractory. The first injection gave a 50 per cent decrease in the sodium excretion while it gave a 37 per cent increase in potassium and an 8 per cent increase in ammonia.

Only one dog (7 kg.) was tested and that became refractory (fig. 1). This animal had been previously injected subcutaneously as the chart indicates.

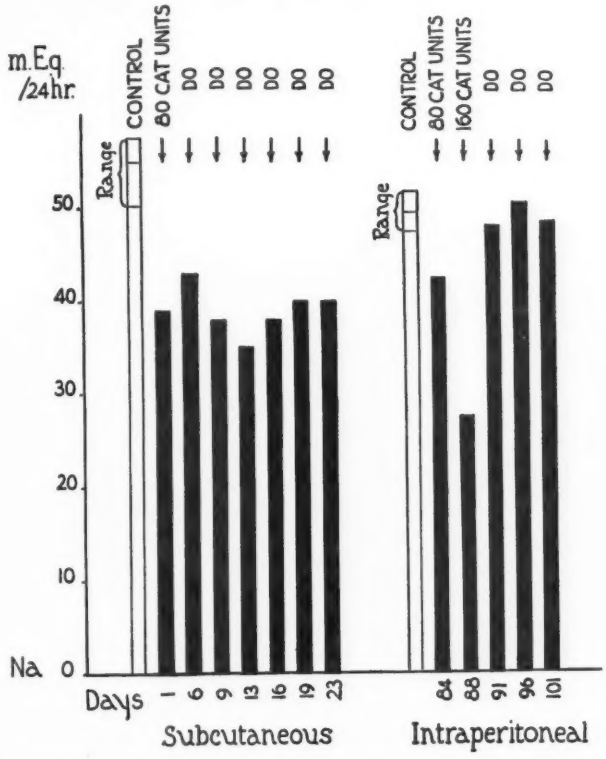


Fig. 1. Sodium excretion of a dog (7 kg.) after the injection of adrenal extract. Refractory state not developed after repeated subcutaneous injection but appeared on the third intraperitoneal injection.

Previous subcutaneous injection might have been a factor in the early development of the refractory state from intraperitoneal injections.

Intravenous Injections of Adrenal Extract. We have employed the intravenous path for injection in the balance of our studies. Adrenal extracts from beef, pig, sheep and horse were tested on dogs refractory to one or more of these extracts. It was found that dogs made refractory to beef extract would give a typical electrolyte response if injected with pig, sheep or horse extract. Similarly dogs refractory to both beef and pig extract responded to sheep and horse extracts and those refractory to pig extract responded to

beef extract. Figure 2 shows the responses observed with various extracts in one of these dogs. This is typical of the responses observed in the 4 dogs studied in this connection. Thus, it is evident that the refractoriness is species specific.

If we used the same number of cat units for injection the initial responses were similar regardless of the species from which the glands were obtained.

The pig was a good subject as far as results were concerned despite the fact that it gained 33.2 kg. during the 7 weeks while the experiments were being carried out. However, it became difficult to handle as its muscular power developed.

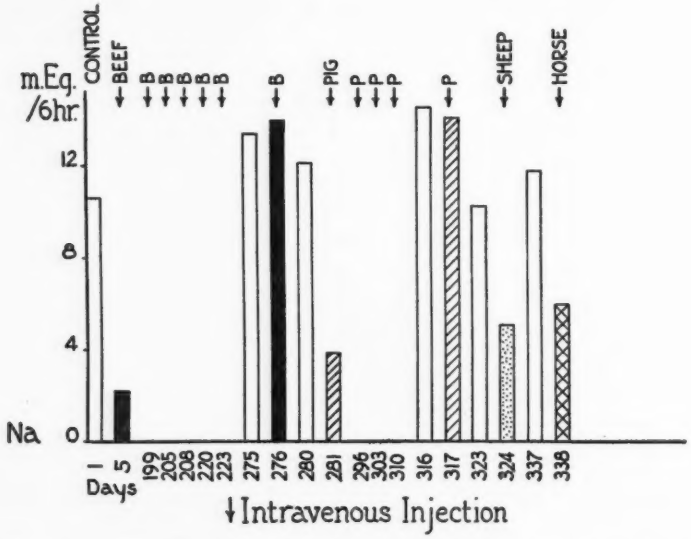


Fig. 2. DEMONSTRATION OF SPECIES SPECIFICITY OF ADRENAL EXTRACTS IN A DOG (10 KG.). Refractoriness after 6 injections of beef extract. However good response to pig extract, this response disappearing after 4 injections. Animal was still responsive to sheep and horse extract.

We first attempted to produce a refractory state by the intravenous injection of extract of pig adrenals. There were 8 injections of 64 C.U. each, beginning at 8 weeks of age and extending over a period of 3 weeks. The control values for sodium (over a 6-hour period), 12 in number, averaged 26.02 m.eq. ranging from 20.09 to 33.2 m.eq. The first injection of pig extract reduced the 6-hour excretion of sodium to 6.5 m.eq. while the eighth injection reduced the excretion to 5.48 m.eq. for a similar period.

We next attempted to produce a refractory state by the intravenous injection of extract of beef adrenals. There were 8 injections of 64 C.U. extending over a period of 3 weeks. The first injection reduced the 6-hour excretion of sodium to 4.74 m.eq. while the eighth injection reduced it to 6.40 m.eq. for a similar period.

Whatever the path of injection, epinephrin in doses corresponding to the maximum content of the extracts was without significant effect on electro-

lyte excretion. This was demonstrated by a number of experiments in 3 dogs.

Frequent microscopic examinations and albumin determinations showed no abnormalities in the urine.

DISCUSSION

Our results explain the failure of Harrop and Thorn to obtain a refractory state. All of their repeated injections were by the subcutaneous path.

The failure of the refractory state to develop from the subcutaneous injection of adrenal extract plus pig serum seems to rule out the possibility of a haptene combination occurring in the blood stream. The experiments with intraperitoneal injections are so few, that they are significant only in that the results were consistent and resembled those with intravenous injection.

Some of the refractory states which have been described previously are only hormone specific (7, 8). In view of this the species specificity of the refractory state to adrenal extract is particularly significant.

The failure of the pig to develop the refractory state may have been due to its age as all other experiments were carried out on adults.

It is desirable that similar studies be conducted with crystalline products. Since the refractory state has been observed in the normal human being following intravenous injections of adrenal extract (3) as well as in the experimental animals it seems better to avoid this path except in an emergency where rapid absorption is required. In this event it would be preferable to use an extract from a species not commonly employed.

SUMMARY

A study was made of the conditions necessary for the development of the refractory state from adrenal extracts in dogs, cats and a pig. All animals were kept on a constant regime including diet. Subcutaneous injections failed to produce the refractory state even after many injections over a long period of time (3 dogs used). There was no indication that the refractory state from intravenous injection was due to haptene formation.

An unquestionable refractory state was produced (in 1 cat and 1 dog) by intraperitoneal injections. The refractory state is species specific. This was shown by experiments on 4 dogs. The pig (a very young animal) failed to develop the refractory state to either pig or beef adrenal extract.

A precaution is suggested for the intravenous injection of adrenal extract in Addison's disease.

We wish to thank Mr. H. J. Spoor for the preparation and assay of extracts. We are also indebted to Doctors George F. Cartland and David Klein for extracts which they kindly furnished.

REFERENCES

1. Thorn, G. W., H. R. Garbutt, F. A. Hitchcock and F. A. Hartman: *Endocrinology* 21: 202. 1937.
2. Harrop, G. A., and G. W. Thorn: *J. Exper. Med.* 65: 757. 1937.
3. Hartman, F. A., L. A. Lewis and G. Toby: *Science* 86: 128. 1937. *Endocrinology* 22: 207. 1938.
4. Cartland, G. F., and M. H. Kuizenga: *Am. J. Physiol.* 117: 678. 1936.
5. Hartman, F. A., and W. D. Pohle: *Endocrinology* 20: 795. 1936.
6. Folin, O., W. B. Cannon and W. Denis: *J. Biol. Chem.* 13: 477. 1913.
7. Thompson, K. W.: *Proc. Soc. Exper. Biol. & Med.* 35: 637. 1937.
8. Young, F. G.: *Biochem. J.* 32: 656. 1938.

THE RELATIONSHIP OF HEMOPOIETIC PHENOMENA TO ENDOCRINE DISORDERS IN WOMEN

E. A. SHARP

From the Department of Clinical Investigation, Parke, Davis & Company

AND H. C. MACK

From the Out-Patient Department, Harper Hospital

DETROIT, MICHIGAN

MORBUS VIRGINEUS was attributed to abnormal menstrual function by Lange in 1554. Naegeli (1), among modern hemopathologists, has commented on the frequent association of ovarian failure and chlorosis. Catapano (2) has reported a favorable effect on anemia in women following treatment with ovarian hormones. In a diagnostic survery of 1000 endocrine cases Rowe and Lawrence (3) observed the blood status of all patients studied. In summary they found that there was no appreciable blood depletion in disorders of the pituitary nor were the red blood elements quantitatively below normal levels in 197 cases of hypogonadism. Their thyroid group, however, showed a 'tendential rather than an established secondary anemia' while anemia was found frequently in cases of biglandular involvement. In contrast to Rowe and Lawrence's observations Falta and Meyers (4) regard blood depletion as characteristic of the late stages of hypophyseal dystrophy.

METHOD OF STUDY

During the past several years approximately 450 anemic women have been studied. An analysis of clinical and laboratory data on this group shows that about 110 patients, or 25 per cent of the series, had concomitant anemia and endocrine disorders. This report covers the diagnostic and therapeutic observations on 68 cases, or only 61.8 per cent of the anemia-endocrine group, since the remaining 42 patients had to be excluded for the usual reasons accruing in the course of attempting to conduct controlled studies in a clinic. A similarity in the symptomatology of the entire group of 110 patients, however, permits a redistribution into three subclasses. While this classification is more or less arbitrary its use insures a clarity of presentation.

Group 1. Age: Menarche to 24 years. Primary Pituitary-Thyroid Deficiency. In these the menarche initiated hypermenorrhea, polymenorrhea or continuous uterine bleeding. Endometrial hypertrophy with or without polyposis is the rule. The basal metabolic rate in the majority is subnormal. Observations on the status of gastric hydrochloric acid are insufficient to indicate a tendency. The anemia is moderate to severe, usually normocytic and rarely hypochromic. Purpura is common accompanied by a prolonged or normal bleeding time. Thrombocytes may be normal or depleted

as an exhaustion phenomenon. Less frequent manifestations are eunuchoid habitus, hypertrichosis, acne, male hirsuties and a familial history of 'bleeders.'

Group 2. Age: 25 to 39 years. Secondary Thyro-Ovarian Failure. This follows systemic disease, pregnancy, malnutrition, overwork, et cetera. Hypermenorrhea or continuous bleeding are most common. Oligomenorrhea and amenorrhea may prevail or alternate with profuse, prolonged uterine bleeding. Gastrointestinal complaints are usual. Cystic ovary and ovariectomy are frequent in the history. Serum phosphorus is usually at the lower limits of normal. Hypochromemia, microcytosis, thrombocytopenia and purpura are manifested, as are achlorhydria, koilonchia, brittle hair, dry skin, pallor, dysphagia and glossitis.

Group 3. Age: 40 years to Menopause. Premenopausal Stage. The age period for this group is indefinite. Doubtless some of the subjects in group 2 merge into the premenopausal class before reaching the minimum age limit of this group. The general symptoms are prolonged and copious uterine bleeding accompanied by anemia. Hypochromemia and microcytosis are common. Psychoneurosis, vasomotor instability, bizarre appetite, hypertension and other exocrine disturbances are seen. There is usually a history indicating past or present thyroid and ovarian failure.

Salient Features of the Anemia

Twenty-four patients, or 35.2 per cent of the total of 68 cases in this report, were between the ages of 13 and 24 years. Six of the 24 had moderate erythrocytopenia upon admission while the remaining 18, or 75 per cent of this group, had severe anemia. Purpura was elicited in about half of this group by the use of a pressure of 100 mm. of mercury for 5 minutes. Although 17 of the 24 cases in this class were menorrhagic there was no constancy in respect to their coagulation and bleeding times. When purpura was elicited the bleeding time usually was prolonged. Thrombocytopenia usually accompanied and followed severe bleeding episodes; in these cases the platelets were found to be poorly structured and below normal in diameter. Microcytosis and hypochromemia were infrequent in group 1, the majority being normochromic and normocytic. Patient *Bo.*, a negress, age 15, showed the sickling phenomenon and is reported in detail elsewhere (5).

In groups 2 and 3 the average red blood cell and hemoglobin levels were appreciably lower on admission than were the average levels for group 1. Group 2 resembled group 1 in that the predominant blood pattern in both was normochromic and normocytic in contrast to the predominance of microcytosis and hypochromemia in the older patients in group 3. Collectively, the average blood and hemoglobin levels show a greater depletion in the two older groups.

The status of hydrochloric acid secretion in this series is significant (table 3). It is unfortunate that the younger patients in group 1 failed to cooperate satisfactorily in efforts to obtain evidence on this item but of the 12 cases studied only 2 were achlorhydric, 3 were hypochlorhydric and the remaining 7 showed normal production of gastric hydrochloric acid. There is an extreme reversal from this status when the findings in groups 2 and 3 are reviewed. Complete achlorhydria (alcohol-histamine test meal) was found in 30, or in 70 per cent of the 42 patients in groups 2 and 3, on whom the observations were made. The incidence of achlorhydria between groups 2

and 3 was comparable, also, there being 17 and 11 instances, respectively. Normal secretion of hydrochloric acid and hypochlorhydria occurred in about the same frequency in groups 2 and 3.

Surveyed as a whole group, with the anemic state as a common denominator, there are several important inferences to be derived from the foregoing data. Primarily, the younger patients (group 1) appear to exhibit a better hemopoietic response to their blood loss, since there is no reason to believe that the menorrhagia in this group was quantitatively less severe than in the two older series of patients. Secondly, the high incidence of complete achlorhydria in the patients of mature years implies either that the secretory function of the gastric mucosa is impaired by anemia, thereby creating the phenomenon described by Apperly (6) as 'anemic achlorhydria,'

TABLE 1. PRETREATMENT RED BLOOD CELL LEVELS OF 389 ENDOCRINE CASES

Endocrine entity	Erythrocytes, mil. per cu. mm.						Totals
	3.0 to 4.0		4.0 to 4.5		Above 4.5		
	M	F	M	F	M	F	
Hypopituitarothyroidism	1	5	7	39	14	22	88
Hypothyropituitarism	0	10	7	31	3	22	73
Hypothyroidism	1	18	2	26	10	14	71
Hypogonadism	0	13	1	22	0	15	51
Hypopituitarism	1	9	3	17	2	13	45
Hyperthyroidism	0	4	2	20	7	12	45
Hyperpituitarism	0	0	1	3	4	3	11
Addison's Disease	1	1	2	0	1	0	5
Totals	4	60	25	158	41	101	389
	64		183		142		

By courtesy of Dr. R. L. Schaefer.

or the exciting cause of the anemic state directly abolishes also the physiologic reaction which is responsible for the production and secretion of hydrochloric acid in the ventriculus.

Individually there were many interesting blood patterns in this series of patients. In some instances the shift from normal to prolonged bleeding time and the periodic variability in the thrombocyte counts defied interpretation. In the bleeding cases particularly, the quantity of blood lost and the duration of the hemorrhagic state would be expected to influence profoundly the hemopoietic pattern. Viewed casually, the hemologic and clinical symptomatology in many of these cases would be sufficient for a diagnosis of 'iron deficiency anemia.' In our opinion, however, a negative iron balance is only one feature of the syndrome under consideration in this report.

Anemia and Endocrine Correlations

Prior to arriving at a definite conviction that an endocrine disorder and an anemia in women constituted a clinical syndrome, an attempt was made to distinguish between the patients who might respond to antianemic or endocrine therapy respectively. After several years' observation it became

evident that those classified as 'anemia' would respond hemopoietically when treated with various antianemic substances, vitamins and minerals, but a tendency to relapse was common. Further, those selected initially for endocrine therapy exclusively showed slow general improvement but only in exceptional instances was any appreciable hemopoietic response observed. While appropriate therapy raised the basal metabolic rates, controlled functional uterine bleeding and in many cases restored menstrual rhythm, re-

TABLE 2. PRETREATMENT LEVELS OF ERYTHROCYTES AND HEMOGLOBIN IN 68 CASES OF ANEMIA-ENDOCRINE SYNDROME IN WOMEN SHOWING DOWNWARD TREND AS AGE INCREASES

Hb. %	R.B.C. (mil.)	Menarche to 24 yr., Group 1 (24 cases)	25 to 39 yr., Group 2 (25 cases)	40 yr. and over, Group 3 (19 cases)
100	5.0			
90	4.5		o	oo
80	4.0	++ ++o oo+ o+o+o	o o+o+	o+ o o
70	3.5	o++oooo ooo ++	o+oo oo ooooo	o+o o
60	3.0	+++ o+o++ o+ooo	++ o o+ ++oo	o ++o+ ++ o
50	2.5	oo + +	o+ o++++o + +	+o+ ++
40	2.0	++ o	o+++o o+++	++ +oo o o+++o
30	1.5	o + +	+ ++	+o o+
20	1.0		o+	

o, Erythrocytes; +, Hemoglobin.

lapses were about as frequent in the endocrine series as in the group treated specifically for anemia.

Logically, the next step was to employ combined antianemic and endocrine therapy. This procedure was adopted. The data, therefore, reported in tables 5, 6 and 7 represent the results obtained by increasing the scope of treatment.

As regards the correlations between co-existant anemia and endocrine disturbances in women, a brief discussion of a few known facts will be helpful. It is accepted that hemorrhage is an exciting cause of anemia. Various

TABLE 3. DETERMINATION OF GASTRIC SECRETION OF HYDROCHLORIC ACID
(Anemia-Endocrine Group)

Group	Achlorhydria	Hypochlorhydria	Normal HCL	Not Done
1	2	3	7	12
2	17	4	2	2
3	11	5	3	0
Totals	30	12	12	14

investigators (7, 8, 9, 10, 11) have studied the effect of menstruation on the erythrocyte and hemoglobin levels in normal subjects. These observers found no appreciable quantitative change during the period of flow, although there was a tendency to lower levels of the red blood elements during the menses with full recovery post-menstrually.

Since hypermenorrhea, polymenorrhea or continuous uterine bleeding are so frequent in the above described anemia-endocrine groups, the effect of various degrees of menstrual blood loss on the peripheral red blood elements cannot be as clearly defined as for the normal subject. Scarcely without exception, however, in the untreated patient each bleeding episode tended to bring about a decrease in red blood cells and hemoglobin. It was noticeable, also that an anemia associated with amenorrhea, oligomenorrhea, normal menses and thyroid disturbance without menstrual abnormality, showed neither a tendency to progress rapidly nor to improve appreciably until treatment was instituted. This comment is made in order to deny a frequently expressed view that non-pernicious anemia displays a tendency toward spontaneous recovery, particularly when basal conditions of living might undergo a favorable change as the result of medical supervision. The majority of the patients in this series were under care and treatment during the worst period of the depression. Dietary histories confirmed beyond doubt that undernutrition was the rule. When the basal living conditions were improved through the assistance of various social agencies, untreated control cases showed only general physical improvement. From observation of a large number of cases of anemia under such conditions of control it is the opinion of one of us (E.A.S.) that a chronic anemic state tends to progress unless therapeutic measures are directed specifically toward the elimination of the cause, amelioration of associated disease, and most important, supplying medicament that will stimulate hemopoietic function.

While the immature patients in group 1 did not show as severe anemia

TABLE 4. BASAL METABOLIC RATES
(Anemia-Endocrine Group)

Group	Below minus 10	Minus 10 to 0	0 to plus 10	Above plus 10	Not Done
1	12	5	0	0	7
2	12	6	0	2	5
3	6	3	2	2	6
Totals	30	14	2	4	18

as that found in groups 2 and 3, at the same time functional uterine bleeding of the post-adolescent patient may rapidly induce a grave anemic state, as exemplified by the following case:

Patient *Ca*, group 1, initially was controlled for several months by the use of 300 to 400 R.U. of the anterior pituitary-like hormone weekly. When endocrine therapy was discontinued bleeding again ensued. She was admitted to the hospital eventually in a grave anemic state. Transfusions immediately and antianemic and endocrine therapy later brought about complete recovery.

Therapeutic Measures

The anterior pituitary-like hormone in doses of 200 to 1000 R.U. should be given daily until uterine bleeding of immaturity is controlled. Thyroid extract to tolerance when indicated and iron salts are essential elements of the therapeutic plan for this group, as well as for groups 2 and 3, when signs and symptoms indicate an appreciable degree of thyroid deficiency and mineral imbalance. The bleeding of maturity as well as the amenorrheic state encountered at any age is an indication for theelin in conjunction with the anterior pituitary-like hormone. Curettement may be indicated at the outset and particularly when endometrial polyposis is present. Bed rest is desirable for patients having the severe periodic or continuous types of bleeding, but the majority can be treated successfully, however, without this approved collateral of therapy.

When the menstrual rhythm has been restored to a sequence resembling a cycle it will be found, even in the course of effective therapy, that periodic menstrual blood loss will reduce the hemoglobin and erythrocytes in many cases. Even after several months of adequate treatment the entire hemopoietic improvement may be dissipated as a result of a few days profuse flow. When this occurs, the premenstrual blood levels can only be restored by persevering in the treatment of the endocrine as well as the hemopoietic deficiency. Figure 1 illustrates the detrimental effect of metrorrhagia on the anemic state. Despite intensive treatment this patient's blood-forming system did not show a tendency to compensate for the menstrual loss until the 4th month of treatment.

The correction of many menstrual disturbances by the judicious use of thyroid extract is too well known to warrant more than a brief mention. In this series thyroid extract alone might have ameliorated the condition in some instances but it is believed that such relief would have been temporary had not collateral endocrine and antianemic treatment been supplied. Iron is known to be beneficial in certain cases of abnormal menstrual bleeding associated with anemia but in our opinion failure to employ the gonadotropic or estrogenic hormones when indicated, in conjunction with thyroid extract and antianemic substances containing protein, is comparable to treating diphtheria with gargles.

It may develop eventually that diagnostic acumen will permit a fine distinction between disorders of monoglandular and those of polyglandular origin. Attainment in this respect would facilitate, of course, intelligent use

TABLE 5. GROUP 1. PRIMARY PITUITARY-THYROID DEFICIENCY. AGE, MENARCHE TO 24 YEARS

Patient	Age, yr.	Gastric Acid and Hemologic Status					Endocrine Aspects			Therapy		Remarks
		Type of Anemia	Admission		Discharge		Signs and Symptoms	B.M.R.	Age of Menarche, yr.	Anemia	Endocrine	
			RBC. mil.	Hb. %	RBC. mil.	Hb. %						
1 *Fo*	14	Achlorhydric Hypochromic	3.7	68	4.9	93	Metrorrhagia.	−16	13	I.S.C., 2 gm. daily (9 mo.)	None	Menses normal B.M.R. +32.
2 *Ca*	16	Normal HCl Normocytic	4.0	85	4.7	97	Male hirsuties, purpura, continuous uterine hemorrhage, endometrial hyperplasia, polypi, acne.	− 7	15	I.S.C., 4 gm. da.; Transfusions	Antuitrin-S, 300 R.U. weekly	Bleeding controlled, severe hemor. curettage, amenorrhea.
3 *Po*	16	Normocytic	3.3	64	—	—	Pigment, thighs, axillae, abdomen, continuous uterine hemorrhage.		15	None	Antuitrin-S, 600 R.U.	Bleeding controlled period of observation inadequate.
4 *Dr*	16	Normocytic	3.8	78	4.8	92	Amenorrhea alternating with oligomenorrhea.	− 8	14	Ventriculin, 10 gm. daily	None	Menses normal after 1 mo.
5 *Mi*	20	Normocytic	3.2	66	4.9	90	Polymenorrhea, 2 to 3 yr., hyperthyroid, (operation) psychoneuroses.	−25	13	Ventriculin, 10 gm. daily	None	Thyroidectomy, improved after 3 mo.
6 *Jo*	13	Normocytic	3.7	77	4.7	90	Mother and sister bleeders, epistaxis, uterine hemorrhage since menarche.		13	I.S.C., 3 gm. daily	Antuitrin-S, 100 R.U. daily	Bleeding controlled, 1 mo. inadequate period of obs.
7 *Tr*	14	Normocytic	3.3	70	5.0	92	Menorrhagia, hysteria, hypothyroid.	−25	11	Ventriculin, 30 gm. daily	None	Periods became normal.
8 *Ze*	18	Hypochlorhydric Micro-hypochromic	3.1	36	4.8	74	Hypothyroid (?). Menses normal.	−13		I.A.C., 3 gm. daily	None	Returned to school after 1 mo. Obs. 2 yr. later normal.
9 *Ra*	13	Normocytic	4.2	82	4.7	87	Sister died uterine hemorrhage, continuous uterine hemorrhage (pubertal).	−16	12	None	Antuitrin-S, 2900 R.U.; Antuitrin-Gonado., 600 R.U.; Thyroid, ¾ Gr. daily	Bleeding controlled with 3 weeks, normal menses 1 yr.
10 *Pa*	24	Hypochlorhydric Normocytic	2.8	60	4.8	92	Uterine bleeding (1 yr.) biopsy, congenital endometrium (hist.).	− 7	14	V-I, 10 gm. daily	Antuitrin-Gonado,. 25 R.U. da.; Antuitrin-T, 50 g.p.u. daily	Normal menses, much improved.
11 *Op*	20	Achlorhydric Normocytic	4.4	80	4.9	94	Amenorrhea, nervous headaches, gastric distress.	−16	14	I.A.C., 1 gm. daily	Thyroid, 2 gr. daily	Greatly improved.
12 *Ho*	23	Normal HCL Normocytic	4.0	65	4.7	82	Purpura, metrorrhagia.	−15	14	V-I	Thyroid, 2 gr. daily	Much improved, bleeding controlled 3 mo. treatment.
13 *Br*	14	Normocytic	3.8	58	4.7	82	Acne, hypothyroidism, epilepsy (petit mal).	−25	12	I.S.C.	Theelol, 300 I.U. daily; Thyroid, 2 gr. daily	Mentally improved, B.M.R. increased.

TABLE 5. GROUP 1—*Continued*

Patient	Age, yr.	Gastric Acid and Hemologic Status					Endocrine Aspects			Therapy		Remarks
		Type of Anemia	Admission		Discharge		Signs and Symptoms	B.M.R.	Age of Menarche, yr.	Anemia	Endocrine	
			RBC. mil.	Hb. %	RBC. mil.	Hb. %						
14 Ga	23	Microcytic	3.7	80	4.7	88	Amenorrhea, oligomenorrhea.			V-I, 10 gm. daily	None	Uncooperative.
15 Bo	15	Macrocytic Sickle cell	2.0	46	—	—	Pubertal uterine hemorrhage, continuous.			None	Theelin, 1000 I.U.	Uncooperative.
16 Ma	23	Normal HCL Normocytic	4.0	89	4.8	96	Endometrial hyperplasia (biopsy), metrorrhagia (1 yr.).			D.C.P.; Dietary	Theelin; Antuitrin-S	Some improvement for several months curettage.
17 Sm	24	Normal HCL	3.6	70	3.6	77	Dysmenorrhea, menorrhagia.	+ 6	13	I.A.C., 2 gm. daily	Snake Venom; Antuitrin-S; Pituitrin; Antuitrin-Gonado., 150 R.U. weekly	Unimproved, adequate therapy.
18 Zw	21	Normal HCL Microcytic	3.2	47	4.8	96	Metrorrhagia, 1 yr., cystic ovary (?)	−17	15	V-I, 5 gm. daily	Antuitrin-S, 600 R.U. weekly; Thyroid, 2 gr. daily	Menses and blood normal, 3 mo. therapy.
19 An	17	Normal HCL Normocytic	4.4	88	4.7	88	Obesity, trochanteric padding, amenorrhea alternating oligomenorrhea (2 yr.)	−13	13	None	Thyroid, 1 gr. daily; Theelin, 6000 I.U. weekly	Body contour improved, observation continued
20 So	13	Normal HCL Normocytic	2.9	62	4.6	88	Uterine bleeding (3 mo.), enlarged thyroid.	−10	15	I.S.C., 3 gm. daily	Antuitrin-S, 600 R.U. weekly	Bleeding controlled, normal menses.
21 Br	13	Hypochlorhydria Normocytic	3.2	55	4.6	82	Oligomenorrhea and uterine bleeding (2 mo.).		13	I.A.C., 2 gm., V-I, 10 gm. daily	Antuitrin-S, 300 R.U. weekly	Still under treatment.
22 An	17	Normocytic	3.8	86	4.3	88	Oligomenorrhea, dysmenorrhea.	−14	11	V-I, 3 gm. daily	Antuitrin-S, 200 R.U. weekly; Thyroid, 1 gr. daily	Gain in weight, 6 mo., still under study.
23 Joh	14	Microcytic	3.9	64	4.1	76	Alternating amenorrhea and uterine bleeding (2 yr.)	−18	13	I.A.C., 1 gm. daily	Antuitrin-S, 200 R.U. weekly; Thyroid, 2 gr. daily	D & C prior to admission. Improved on therapy.
24 Re	17	Normocytic	1.9	32	4.2	80	Uterine bleeding (3 mo.), amenorrhea (6 mo.) after D & C. then resumed bleeding.		13	Transfusions; V-I, 10 gm. daily	None	Still under observation after D & C.

I.S.C. = Iron Sodium Citrate (Naferon). I.A.C. = Iron Ammonium Citrate. V-I = Ventriculin with Iron. D.C.P. = Dicalcium Phosphate.

The digestion of I.U. for theelol used here is now obsolete, it refers to theelol in its equivalent to theelin.

of specific endocrine therapy. But for the present, at least, the physiogenic complexity of the anterior pituitary-thyroid-gonad interrelationship is such

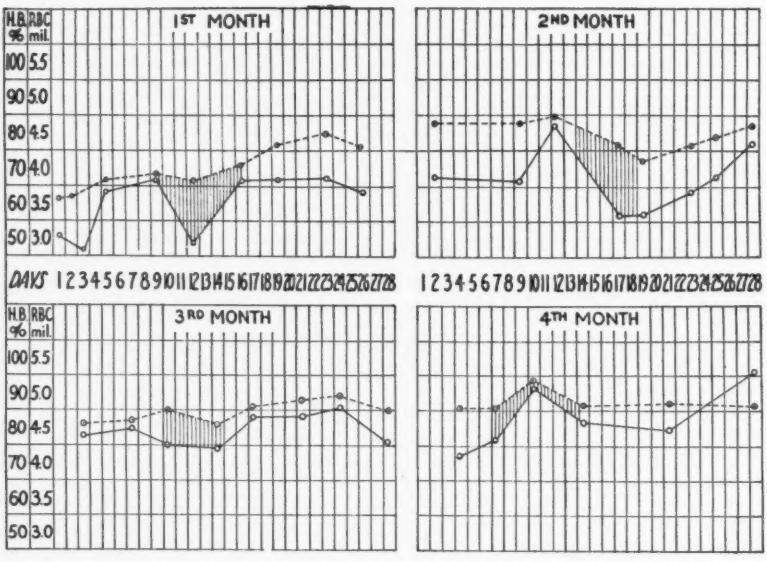


Fig. 1. Hemograph showing the detrimental effect of menstruation on the anemic state in women. Normal menses may dissipate partially or entirely the hemopoietic gain induced during the early phase of treatment, as in the case cited above.

that only by trial and error can the most effective regimes of endocrine therapy be selected.

DISCUSSION

Realizing that clinic patients may show a slightly lower physical standard than private patients, pretreatment hemologic data on 389 endocrine cases from a private practice[1] were consulted. These data (table 1) denote a constant incidence of anemia irrespective of age, sex or endocrine disorder. Furthermore, the frequency of an anemic state in the hypogonadal group is particularly remarkable.

Hubble (12) several years ago reviewed the clinical and experimental evidence relating to the effect of incretory disturbance on hemopoiesis. His collected data showed that the thyroid hormone stimulates production of red blood cells and lymphocytes and depresses the output of granulocytes, while the suprarenal cortical hormone stimulates granulocytopoiesis and also possibly erythropoiesis. Further, Hubble found support for the theory that the basophil cells of the anterior hypophysis induce increased production of all types of blood cells. Moehlig (13) has reported that anterior pituitary basophilism is characterized by a tendency to erythremia and that less constantly there is found a 'polymorphonuclear leukocytosis.' It would be ra-

[1] Through the courtesy of Dr. R. L. Schaefer of Detroit.

TABLE 6. GROUP 2. SECONDARY THYRO-OVARIAN FAILURE. AGE 25 TO 39 YEARS

Patient	Age, yr.	Gastric Acid and Hemologic Status					Endocrine Aspects			Therapy		Remarks
		Type of Anemia	Admission		Discharge		Signs and Symptoms	B.M.R.	Age of Menarche, yr.	Anemia	Endocrine	
			RBC. mil.	Hb. %	RBC. mil.	Hb. %						
1 Ro	28	Achlorhydric Hypochromic	3.8	68	4.8	96	Prolonged uterine bleeding 2 yr.	−13	15	I.S.C., 2 gm. da.	Antuitrin-S, 300 R.U. weekly	Recovered rapidly, normal menses.
2 Fi	26	Achlorhydric Normochromic	2.4	53	4.5	84	Since menarche, right ovariectomy, oligo- and polymenorrhea.	+20	15	I.S.C., 4 gm. daily	Theelol, 1000 I.U. daily	Bleeding controlled, polyposis.
3 Gre	30	Achlorhydric Microcytic	3.2	46	4.8	57	Hypothyroid, uterine hemorrhage.	−41	18	I.S.C., 4 gm. daily	Thyroid	Much improved, menses normal.
4 Gri	31	Purpura Normocytic	1.2	20	0.71	16	Polymenorrhea since menarche, severe hemorrhagic episode.		17	V-I, 30 gm. daily; Transfusions	Antuitrin-S, 200 R.U. daily	Died—hemorrhage.
5 Se	32	Hypochlorhydric Macrocytic (8 yr.)	2.9	30	4.8	96	Enlarged thyroid, hypermenorrhea.	−20	11	I.A.C., 4 gm. daily	Antuitrin-S, 200 R.U. daily	Improved 2 yr., died 1 yr. later.
6 Ko	38	Achlorhydric, microcytic, hypochr.	3.8	56	4.9	93	Thyroadenoma, hypermenorrhea.	− 8 − 5	13	I.S.C., 4 gm. da.; Vitamin B	Theelin; Thyroid, ¾ gr. t.i.d.	Improved, 5 mo.
7 Ba	30	Achlorhydric Normocytic	4.0	80	5.1	100	Amenorrhea, oligomenorrhea.	+32	11	I.A.C., 4 gm. daily	Antuitrin-S, 600 R.U. weekly	Improved, became pregnant.
8 Wo	29	No record HCL Microcytic	3.3	57	4.6	65	Alternating amenorrhea and oligomenorrhea since menarche.		15	I.A.C., 4 gm. daily	Antuitrin-S, 400 R.U. weekly	Improved, 5 mo.
9 Wr	31	Achlorhydric Hypo-normocytic	3.5	55	5.1	100	Amenorrhea, oligomenorrhea.	− 8		V-I, 20 gm. daily	Theelol, 800 I.U. daily	Much improved, menses established, became pregnant.
10 Ca	38	Hypochlorhydric Microcytic	3.6	56	4.7	90	Ovariectomy 8 yr., obese, menorrhagia 8½ yr.			V-I, 20 gm. daily; Vitamin B.	Antuitrin-S	Improved.
11 De	34	Hypochlorhydric Normocytic	4.3	58	4.6	86	Ovariectomy 8 yr., irregular menses.	0	11	I.S.C., 3 gm. daily	None	Did not cooperate.
12 Ba	37	Achlorhydric Macrocytic	3.5	42	5.0	100	Thyroidectomy, oligomenorrhea, 12 pregnancies.	−10	14	I.S.C., 4 gm. daily	Theelin, 2000 I.U. weekly; Thyroid, ½ gr. daily	Normal after 4 mo. Observed 14 mo.
13 Mo	28	Hypochlorhydric Normocytic	4.2	82	4.7	87	Ovarian cysts, splenomegaly, purpura, thrombocytopenia, menorrhagia, oligomenorrhea.	−11	16	Viosterol; iron; calcium	Antuitrin-S; theelin; thyroid	No improvement in 7 mo., still under treatment.

TABLE 6. GROUP 2—*Continued*

Patient	Age, yr.	Gastic Acid and Hemologic Status					Endocrine Aspects			Therapy		Remarks
		Type of Anemia	Admission		Discharge		Signs and Symptoms	B.M.R.	Age of Menarche, yr.	Anemia	Endocrine	
			RBC. mil.	Hb. %	RBC. mil.	Hb. %						
14 Re	27	Normocytic Achlorhydric	2.8	42	5.1	44	Partial ovariectomy (22), uterine hemorrhage 10 mo.	−10	16	I.A.C., 4 gm. daily	None	Under observation 1 yr., did not improve.
15 Em	33	Achlorhydric Macrocytic	3.8	45	5.5	96	Para 11, amenorrhea.	− 2		I.S.C., 4 gm. daily	None	Menstruated second mo., much improved.
16 Re	27	Achlorhydric Normocytic	4.5	60	4.8	87	Enlarged thyroid, uterine hemorrhage.		11	V-I	Antuitrin-S	Became pregnant, dropped from observation.
17 Qr	34	Achlorhydric Hypochromic	3.7	60	4.9	86	Obesity, uterine hemorrhage.	−12	13	I.A.C., 4 gm. daily	Antuitrin-S, 200 R.U. weekly	Much improved.
18 Ha	34	Achlorhydric Hypochromic	2.8	50	5.1	88	Uterine hemorrhage, 4 yr.		12	I.A.C., 4 gm. daily	None	Improved, 1 yr. therapy.
19 Bai	33	Hypochromic Achlorhydric	3.5	43	5.0	96	Thyroadenoma, melancholia, inanition, metrorrhagia, 1 yr., psychoneuroses.	−12 −11	15	I.A.C., 3 gm. daily	None	Suicide, 1932.
20 Gr	38	Achlorhydric Microcytic	3.2	46	4.8	57	Menomenorrhagia 2 yr.	+11	18	I.S.C., 4 gm. daily	None	Improved.
21 Ta	38	Achlorhydric Normocytic	3.0	68	4.7	92	Exophthalmos—enlarged thyroid, menorrhagia.	−33	16	V-I, 20 gm. daily; I.A.C., 1 gm. da.	None	Bleeding and anemia controlled.
22 Ku	35	Achlorhydric	2.3	37	5.3	98	Ovariectomy 1929. Bleeding, hysterectomy, 1931.	−17	15	V-I, 10 gm. daily; I.A.C., 3 gm. da.	None	Recovered.
23 Wa	28	Achlorhydric Microcytic	2.3	32	3.7	81	Profuse menses, undernutrition.	−20	12	V-I, 2 gm. daily	Thyroid	Normal menses, anemia refractory.
24 Ch	28	Normal HCL Normocytic	3.5	63	4.6	90	Menorrhagia			ISC., 4 gm. daily	None	Improved.
25 Gi	35	Normal HCL Normocytic	3.5	70	4.3	92	Hypothyroidism, hypogonadism, eunuchoid habitus, frigidity.	−21	14	I.S.C., 2 gm. daily	Theelin, 6000 I.U. weekly	Greatly improved treatment continued.

tional to assume, therefore, that hemopoietic depression might readily succeed hypofunctional states of the thyroid and anterior pituitary glands, since their incretions may take an active part in maintaining hemopoietic balance under normal conditions.

Several observers are inclined, not without justifiable reason, to attribute the frequency of anemia in women to a constitutional weakness of the blood-forming organs. Wright (14) asks the question and then expresses the opinion that while constitutional inferiority in the hemopoietic system of women has not been proved, there are differences in habits and environments 'which may have an influence.' Witts (15) points out that the reproductive function in women places an exceptional burden on their hemopoietic system which may account for the greater incidence of hypochromic anemia in women than in men. Waugh (16) emphasizes a predisposition in women to develop hypochromic anemia with achlorhydria before the menopause. Graves (17) asserted that thrombocytopenic purpura hemorrhagica is three times as common in women as in men and not infrequently is a cause of menorrhagia in young women. Haden and Singleton (18) have reported disturbances of menstruation in cases of hypochromic anemia, as have Gray and Wintrobe (19) and others.

Of significance in the anemia problem in women are the studies of Gillett, Wheeler and Yates (20), Barer, Fowler and Baldridge (21) and Fowler and Barer (22) which show an enormous iron loss incident to menstruation. Fowler and Barer found the iron loss to range between 3.5 and 450 mg. (average 120 mg.) in the menstrual blood of 10 anemic women who considered their menses normal. Barer, Fowler and Baldridge reported that the menstrual loss of blood iron in a group of 50 normal women varied from 3.84 mg. to 78.4 mg. Since the daily intake of iron in food is comparable to the minimal loss cited by these observers the obvious inference is that a great disparity between intake and menstrual loss of iron exists in some women. Further, the subjects showing the greatest disparity would be expected to develop hypochromemia when and as the iron reserves of the body become exhausted. These deductions do not dispose of the subject of iron deficiency anemia in women, however, inasmuch as some biologic factor evidently determines the threshold for iron excretion during menstruation, since the quantity of iron lost by 50 normal women showed a wide variation.

Among the frequent comments in current literature on anemia in women there is a paucity of data on the endocrine status of the patients studied. The assumption is warranted, then, that glandular disorders were overlooked, since the incidence of an anemia-endocrine imbalance in our group of about 450 anemic women is far too great to be casual.

Can it be assumed that the menorrhagia of hemorrhagic thrombocytopenic purpura is primarily the result of hemopoietic disturbance? The frequency of purpura with and without severe thrombocytopenia in advanced endocrine disturbances in the female warrants the suggestion that there may be a very close relationship between glandular balance and the hemolytopoietic function.

Chlorotic anemia in women between postadolescence and the menopause has been widely discussed in recent literature. It would be enlightening to know, however, whether iron deficiency is the only etiologic factor which gives rise to hypochromemia. Bunge in 1889 asserted that the female organism, beginning even at puberty, puts aside reserves of iron for the future needs of reproduction and that withdrawal of this iron from circulation is related to chlorosis. While iron deficiency alone would seem to be a causal factor, at the same time experience shows a substantial incidence in women

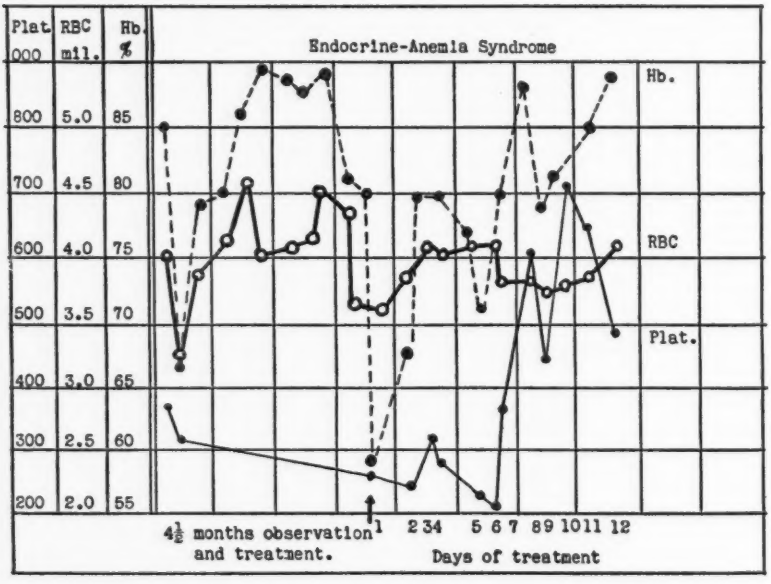


Fig. 2. HEMOGRAPH SHOWING THE BEHAVIOR OF THE RED BLOOD CELLS, PLATELETS AND HEMOGLOBIN ON PATIENT 2. *Ca*, TABLE 5, DURING TWO PHASES OF TREATMENT FOR UTERINE BLEEDING. For a period of 4½ months combined antianemic and glandular treatment induced specific and general improvement. The second period of treatment was necessitated by a sudden severe hemorrhage from the uterus after which endocrine and antianemic therapy were resumed. This patient has remained normal for 2 years.

of a chronic anemic state morphologically of the normochromic type. In this form, there is usually a balanced reduction of erythrocytes and hemoglobin which may manifest normocytic, microcytic and occasionally a macrocytic erythrocytopenia. Presumably, then, chlorotic anemia is conditioned by iron impoverishment while in the chronic normochromic type the iron balance is of less consequence in etiology than other factors. Minerals other than iron, blood building proteins and vitamins may, therefore, be involved in deficient erythrogenesis. Finally, and because of the intimate association narrated in this report, it is proposed that endocrine disorders are possible causative factors of the anemic state.

Chloranemia which occurs in women during the reproductive period almost exclusively is attributed in numerous contemporaneous reports to

TABLE 7. GROUP 3. PREMENOPAUSAL STAGE. AGE 40 YEARS TO MENOPAUSE

Patient	Age, yr.	Gastric Acid and Hemologic Status					Endocrine Aspects			Therapy		Remarks
		Type of Anemia	Admission		Discharge		Signs and Symptoms	B.M.R.	Age of Menarche, yr.	Anemia	Endocrine	
			RBC. mil.	Hb. %	RBC. mil.	Hb. %						
1 McG	41	Achlorhydric Hypochromic	4.9	51	5.4	97	Obese (210 lb.), para 10, menomenorrhagia.		12	I.A.C., 2 gm. daily	Thyroid Ext., 2 gr. daily	Rapid improvement.
2 Gi	40	Achlorhydric Hypochromic	3.2	63	4.8	95	Thyroidectomy 1920, (partial ovariectomy), sterility, menorrhagia.		13	V-I, 10 gm. daily	None	Endometrial hyperplasia. Improved.
3 Ba	42	Hypochlorhydric Macrocytic	2.0	33	4.3	81	Uterine hemorrhage, endometrial hyperplasia.	+ 8	12	V-I, 20 gm. daily	Antuitrin-S, 200 R.U. weekly	Bleeding not controlled, anemia slightly improved.
4 Ha	42	Hypochlorhydric Macrocytic	4.0	68	4.5	93	Anxiety, neurosis, obesity, uterine hemorrhage.	−23	12	I.S.C., 2 gm. daily	Theelin 2000 I.U. weekly; Thyroid, 2 gr. daily	Bleeding controlled, anemia improved.
5 Do	42	Achlorhydric Normocytic	.21	32	4.6	92	Bleeding hemorrhoids, anemia 5 yr., (abnormal sweets), amenorrhea, epilepsy.			I.S.C., 3 gm. daily	None	Anemia improved.
6 McR	40	Hypochlorhydric Microcytic	3.4	48	3.7	75	Cervical polypi, menomenorrhagia.	−20	17	I.A.C., 4 gm. daily	Theelin, 1000 I.U. weekly.	Not much improvement after 3 yr. therapy.
7 Wi	43	Achlorhydric Microcytic	3.4	50	4.7	94	Uterine hemorrhage.	− 8	14	I.A.C., 4 gm. daily	Thyroid Ext., 2 gr. daily	Cessation of bleeding, anemia improved.
8 Po	47	Normocytic Achlorhydric	4.4	58	4.3	83	Menorrhagia.	+ 9	15	I.S.C., 2 gm. daily	Theelin, 2000 I.U. weekly	Not much improvement in 6 mo.
9 Mi	40	Achlorhydric Macrocytic	2.0	40	4.6	86	Endometrial hyperplasia, biopsy, menometrorrhagia.	−10	13	I.S.C., 3 gm. daily	Antuitrin-S, 300 R.U. daily	Did not cooperate well.

TABLE 7. GROUP 3—*Continued*

Patient	Age, yr.	Gastric Acid and Hemologic Status					Endocrine Aspects			Therapy		Remarks
		Type of Anemia	Admission		Discharge		Signs and Symptoms	B.M.R.	Age of Menarche, yr.	Anemia	Endocrine	
			RBC. mil.	Hb. %	RBC. mil.	Hb. %						
10 We	40	Achlorhydric Normocytic	3.8	42	4.7	94	Thyroidectomy, menses prolonged.	−11 −14	13	I.S.C., 4 gm. daily	Thyroid Ext.	Improved rapidly.
11 Sh	40	Hypochlorhydric Normocytic	3.7	68	4.5	85	Gastrointestinal disorders, hypothyroid, menses profuse.	−12	12	V-I, 20 gm. daily	Theelol, 300 I.U. daily; Thyroid 2 gr. daily	Very much improved in 6 mo.
12 Me	40	HCL normal Microcytic	3.8	46	4.4	96	Psychoneuroses, dyspnea, menses normal (dye).	−25		I.S.C., 4 gm. daily	Thyroid, 2 gr. daily	Irregular therapy, Not cooperative.
13 Gr	44	HCL normal Microcytic	2.8	59	4.9	98	Uterine hemorrhage, alternating amenorrhea.			V-I, 20 gm. daily	None	Improved.
14 Du	45	Hypochlorhydric Macrocytic	3.7	88	4.3	96	Menorrhagia, hypothyroid, gastrointestinal disturbance.	−20		V-I, 20 gm. daily; I.S.C., 4 gm. da.	Theelin; Thyroid, 1½ daily gr.	Improved, 6 mo.
15 Ka	48	Achlorhydric Microcytic	4.0	64	4.8	95	Menorrhagia.	− 6	14	V-I, 10 gm. daily	Theelin, 2000 I.U. weekly	Improved, under observation 5 yr., anemia improved
16 St	48	Achlorhydric Normocytic	2.3	51	4.8	87	Hyperthyroidism.	+32		V-I, 30 gm. daily	None	Improved.
17 Me	44	Achlorhydric Microcytic	4.2	48	4.8	98	Oligomenorrhea, nervous, menorrhagia, vertigo.			I.A.C., 4 gm. daily	Ovarian substance only	Much improved, 3 mo.
18 Ma	40	Achlorhydric Microcytic	2.0	49	5.0	95	Oligomenorrhea, enlarged thyroid, gastric distress.			V-I, 20 gm. daily	None. Early case	Improved rapidly.
19 Mu	44	HCL normal Normocytic	1.7	30	4.5	82	Amenorrhea 6 mo., menorrhagia, hyperplastic endometrium, hyperthyroidism.	30+48+ 11+ 52+26+	13	Vitamin B; V-I	Theelin, 2000 I.U. weekly; Antuitrin-S, 300 R.U. weekly	B.M.R. normal, anemia cured. Bleeding controlled, 1 yr.

I.A.C., Iron and Ammonium Citrate. I.S.C., Iron and Sodium Citrate (Naferon). V-I, Ventriculin with Iron.

'iron deficiency' or to an idiopathic mechanism. It is believed that the high incidence and severity of this anemic state would be modified favorably were pituitary-thyroid-gonad endocrinopathies recognized and treated.

In figure 3 a theoretical conception of the factors involved in the pathogenesis of an anemia-endocrine syndrome is presented. It is conceivable that anemia may be a result of an endocrine disturbance. A perplexing question still arises, however, as to what initiates the disturbed glandular function and how it affects the hemopoietic system. Apperly and Crabtree (23) have

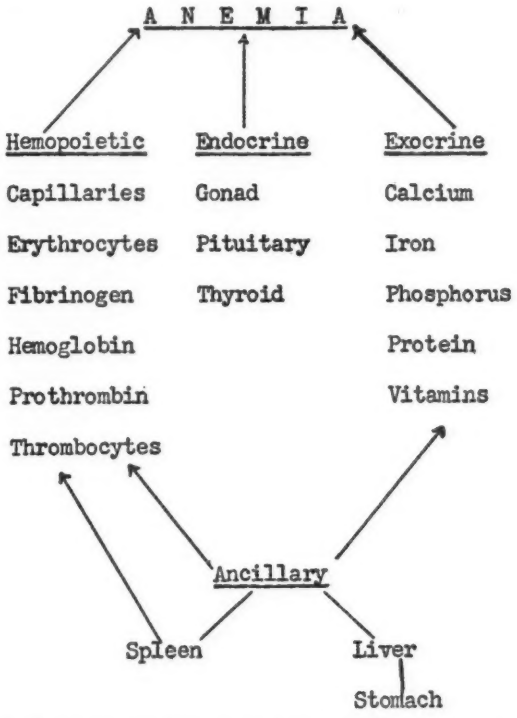


Fig. 3. THIS DIAGRAM PRESENTS THE COMPONENTS OF VARIOUS SYSTEMS SEVERAL OR ALL OF WHICH MAY BE HYPOTHETICALLY INTEGRATED AS DETERMINANTS IN NORMAL HEMOPOIETIC AND GLANDULAR FUNCTION.

demonstrated that a reduction in the secretion of gastric hydrochloric acid occurs when there is a negative acid-base balance. Is achlorhydria then a defense mechanism designed to conserve acid-base? Does glandular dysfunction produce disturbances in the digestive function, both of which in turn induce protein depletion and prevent the assimilation of minerals and vitamins? While the anemic state may develop as a direct result of mineral and vitamin deficiency it is equally acceptable that hemopoietic depression results from acute or chronic blood loss. Hormones must also become depleted through constant loss by hemorrhage.

Since adequate production of hormones and proteins may be denied the organism by deficient intake or assimilation of minerals, vitamins and protein, or both, as well as suffer depletion through constant loss by hemorrhagic processes, the final expression of this impoverishment are exhaustion phenomena of the glandular and blood-forming systems.

Finally, the exciting causes precipitating this course of events must be sought before factual evidence becomes sufficient to permit a distinction between cause and effect.

SUMMARY

It is believed that there is a definite anemia-endocrine syndrome in women. The patients coming under this classification can be divided into 3 age groups: *a*), the immature, menarche to 24 years of age, *b*), the mature thyro-ovarian failure type, 25 to 39 years of age, and *c*), the premenopausal or involutional type, 40 years of age to the menopause.

Definite signs of hypothyroidism, pituitary failure and hypogonadism are common to the syndrome. The anemia is usually normocytic in the adolescent and early adult age groups. Microcytosis and hypochromia appear to be expressions of chronicity of the anemic state, since they occur with greater frequency in middle-aged patients. Achlorhydria is frequent in all three age groups, as are low basal metabolism, purpura, uterine bleeding, gastrointestinal disturbance and psychoneuroses. Simultaneous treatment of the hemopoietic and endocrine disturbances by appropriate medicaments insures a favorable prognosis in a majority of cases.

REFERENCES

1. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, 4th Edition, Springer, Berlin, 1923.
2. Catapano, R.: *Rossequa. di. Clin. terap. e. Sc. Off.* 28: 333. 1929.
3. Rowe, A. W., and C. H. Lawrence: Endocrine Studies, No. 1, Robert Dawson Evans Memorial Reports, 1929, pp. 237, 389, 603 and 719.
4. Falta, W., and M. K. Meyers: Endocrine Diseases, 3rd Edition, Blakiston, Philadelphia, 1923.
5. Sharp, E. A., and E. M. Schleicher: *Am. J. Clin. Path.* 6: 580. 1936.
6. Apperly, F. L.: *Lancet.* 1: 5. 1936.
7. Holler, G., H. Melicher and N. Reiter: *Ztschr. f. klin. Med.* 100: 564. 1924.
8. Detre, L.: *Ztschr. f. d. ges. exper. Med.* 59: 241. 1928.
9. Reich, C., and D. Green: *Arch. Int. Med.* 49: 534. 1932.
10. Rowe, A. W., and M. C. Gaugenty: *J. Lab. & Clin. Med.* 20: 253. 1934.
11. Smith, C.: *Am. J. Physiol.* 114: 452. 1936.
12. Hubble, D.: *Lancet.* 2: 113. 1933.
13. Moehlig, R. C.: *J. Michigan M. Soc.* 34: 263. 1935.
14. Wright, C. B.: *Northwest Med.* 32: 3. 1933.
15. Witts, L. J.: *Practitioner.* 127: 442. 1931.
16. Waugh, T. R.: *Arch. Int. Med.* 47: 71. 1931.
17. Graves, W. P.: Gynecology, 4th Edition, Saunders, Philadelphia, 1928.
18. Haden, R. L., and J. M. Singleton: *Am. J. Obst. & Gynec.* 26: 330. 1933.
19. Gray, L. A., and M. M. Wintrobe: *Am. J. Obst. & Gynec.* 31: 3. 1936.
20. Gillett, L. H., L. Wheeler and A. B. Yates: *Am. J. Physiol.* 47: 25. 1918.
21. Barer, A. P., W. M. Fowler and C. W. Baldridge: *Proc. Soc. Exper. Biol. & Med.* 32: 1458. 1935.
22. Fowler, W. M., and A. P. Barer: *J.A.M.A.* 104: 144. 1935.
23. Apperly, F. L., and M. G. Crabtree: *J. Physiol.* 73: 331. 1931.

A NEW AND IMPROVED METHOD FOR THE PREPARATION OF PINEAL EXTRACT[1]

ARTHUR STEINBERG

From the Philadelphia Institute for Medical Research in the Philadelphia General Hospital

PHILADELPHIA, PENNSYLVANIA

IN A PAPER by Rowntree, Clark, Steinberg and Hanson (1), mention is made of the results obtained with a pineal extract prepared by Hanson. The technique for its preparation is outlined in a subsequent paper by the same authors (2), in which Hanson extracts a pineal picrate with dilute acid (0.2 per cent HCl by volume) and heat, to 90° C. In the same article there is a footnote on the use of an HCl extract of defatted, desiccated pineal glands which was equally biologically effective and much less toxic. Three cc. of the former extract administered to a 100 gm. rat would cause a non-specific protein reaction, characterized by a rapid tremor and lowered body temperature, from which the animal often failed to recover. With the present method of preparation no untoward effects are observed with as much as 20 cc. administered intraperitoneally.

To date there has been no chemical test available, whereby it is possible to determine rapid estimation of potency. However, this is not so important as in the case of thymus extract, as by following the procedure as outlined here, it has been possible to obtain a uniformly biologically potent extract at all times.

Experimental Procedure. The technique of preparing the extract is as follows: pineal glands of beef at killing age are collected in acetone, and the dehydrated, defatted glands are then dried and powdered.[2] Fifty gm. of this dried material is extracted with 1000 cc. of 0.4 per cent (by volume) hydrochloric acid, and heated with constant stirring to 75° C. The material is then allowed to stand for 5 minutes in order to determine if flocculation is complete. At this stage there is considerable variation in the conditions under which the material coagulates. This is accomplished by adding small amounts of powdered sodium carbonate, followed by vigorous stirring until isoelectric point is obtained, when the proteins flocculate and sediment immediately, yielding a clear golden supernatant fluid. The extract may be removed by filtration through coarse filter paper, by syphoning, or by centrifuging, and preserved with 0.2 per cent chlorobutanol. The extract is stored in bulk in

[1] From the Samuel Bell, Jr. Laboratory. This work was aided by grants from the Penrose Fund of the American Philosophical Society. These chemical studies have been made possible through the generosity of the Rockefeller Foundation.

[2] This dry powdered glandular material was generously supplied by Armour & Company.

pyrex flasks with refrigeration at 40° F. Attempts have been made to vary the pH and the temperature without much success.

The extract as prepared for biological use is at all times protein-free, when made according to the method outlined above. There is also no iodine-reducing material, and it shows a negative Millons reaction. Two factors which may be of importance are the ability of the extract to reduce appreciable amounts of potassium permanganate and the mild biuret reaction. The extract has been found to be very stable on ageing and on heating, contains no depressor or pressor substances, and is non-irritating locally.

EXPERIMENTAL PHASES OF THE PINEAL PROBLEM[1]

N. H. EINHORN AND L. G. ROWNTREE
From the Philadelphia Institute for Medical Research in the Philadelphia General Hospital
PHILADELPHIA PENNSYLVANIA

IN PREVIOUS communications (1), attention was called to the accruing retardation in the rate of growth and the acceleration in the rate of somatic differentiation of the offspring when successive generations of white rats (Wistar strain) were given daily intraperitoneal injections of 1 cc. of a potent pineal extract. It seemed likely that if an excess of pineal tissue or extract resulted in a retardation in the rate of growth and acceleration in the rate of development of normal rats that removal of the pineal gland from successive generations of parent rats might result in the opposite effects, i.e., acceleration in growth and retardation in development.

PINEALECTOMY AND PINEAL IMPLANTS

The success attending our experiments involving thymus implants in normal animals, thymectomy, thymectomy in split-test groups, replacement therapy with thymus extract and thymus implants in normal rats (2) led us to undertake a similar group of experiments on the pineal gland in successive generations of rats.

Accordingly, 21-day-old rats were selected from the normal stock animals of the Institute, and the following experiments were started: *a*), pinealectomy, *b*), pinealectomy in split-test groups, *c*), injection of pineal extract in pinealectomized rats, *d*), frequent homologous pineal implants in pinealectomized rats, *e*), homologous pineal implants in normal rats, *f*), control experiments with pineal extract in normal rats. All of the rats were housed and fed under conditions previously described.

The rats were mated, designated by numbers, and their offspring studied. Data on somatic development up to the age of 70 days were recorded daily, and the weights of the rats twice weekly. All of the animals in one litter or part of a large litter were mated and set up as parents of the next generation.

The operations were done under ether anesthesia. A longitudinal incision was made through the scalp exposing the calverium from a point just posterior to the ears and extending to the base of the occiput. With small sharp scissors, a flap of bone consisting of small portions of the occipital and parietal bones was elevated, exposing the dura, and venous sinuses. In most instances, the pineal body could be seen as a small globular mass through the meninges. With blunt tissue forceps, an opening was made through the meninges, the pineal grasped and removed in toto by gentle traction. The bone flap was then returned to its normal position, and the scalp sutured with several interrupted sutures. No incision was closed until it was felt that the entire pineal gland had been removed.

---

[1] From the Samuel Bell, Jr. Laboratory. This work was aided by grants from the Penrose Fund of the American Philosophical Society.

The earliest age at which animals were pinealectomized was 6 days, and the latest 21, with an average age of 10 days. A total of 97 pinealectomies have been done for this series of experiments with a mortality of 5.2 per cent.

In transplanting the pineal gland for the implant investigations, the same technique was used as in the thymus implant experiments previously described (2).

In order to control more accurately the results in the offspring of pinealectomized animals, a study was made of a special group of rats whose parents were subjected to the surgical technic concerned in pinealectomy but in whom the pineal body was not actually removed. A study of 195 rats born in this group of operative controls showed no significant deviation in growth and development from the stock control animals of this Institute.

Effects of Pinealectomy. To date, 6 successive generations of the pinealectomy tests rats have been observed, distributed as follows: 1st generation (F_0) 6, 2nd generation (F_1) 115; 3rd generation (F_2) 155; 4th generation (F_3) 144; 5th generation (F_4) 159; and 6th generation (F_5) 90, making a total of 663 rats in 85 litters of which 544 or 81 per cent survived the 70-day period of observation. The average age at which the first litter was born was 89.5 days, the average period between gestations was 47 days, and the number of animals in each litter varied from 3 to 12 with an average of 7.8 rats per litter.

As a result of pinealectomy in the original group of 6 rats, no abnormality in their growth or development was noted. Likewise, irrespective of the generation concerned, pinealectomy itself exercised no significant effect on the growth and development of the individual animals concerned. This was evident in several instances in which only 2 or 3 animals of a large litter were subjected to operation. The growth and development of the operated animals did not differ materially from that of the unoperated controls of the litter. Therefore, no differentiation has been made between the rate of growth of pinealectomized and non-pinealectomized offspring. The data herein presented refer to and include both the operated and non-operated animals in each generation.

In table 1, *A*, are presented the average weights, at 10-day intervals, of the test animals in each generation. Comparison of these weights with the weights of the operative controls shows that the rate of growth of the test rats showed no definite acceleration or retardation throughout the entire 70-day period of observation. An increase in weight of pinealectomized animals has been described in a small series of animals by D'Amour and D'Amour (3). This finding was not confirmed in our experiments where the rate of growth of the parent rats in each generation was observed beyond the age of 70 days.

Likewise no uniform or noteworthy departure from the normal in the time of the opening of the ears, the eruption of the teeth, the development of fur, the opening of the eyes, the descent of the testes, or the opening of the vagina was noted (table 2, *A*).

Effects of Pinealectomy in Split-Test Groups. Three pinealectomized males were mated to 3 normal females; and 3 pinealectomized females were mated to normal males. The former group was designated the male split-test group, and the latter the female split-test group.

In the male split-test group, a total of 532 rats distributed in 5 generations were studied. In the female split-test group, 489 rats similarly distributed were observed. There was essentially no difference between the average weights of the animals of either of these test groups and the average weights of the operative controls. Similarly, no striking difference was seen

TABLE 1. AVERAGE WEIGHT, IN GRAMS, AT 10-DAY INTERVALS, OF CONTROLS AND SUCCESSIVE GENERATIONS OF TEST RATS

Age, days	Control	F_1	F_2	F_3	F_4	F_5
			A. Pinealectomy Group.			
Birth	4.9	4.8	4.8	4.7	4.7	4.8
10	15.0	14.8	13.0	14.7	14.6	14.7
20	28.5	28.3	28.3	29.4	29.2	28.3
30	49.1	51.6	51.4	51.4	50.7	52.4
40	68.6	74.1	74.8	70.1	71.6	71.9
50	93.5	92.0	92.3	94.6	94.3	92.3
60	113	111	111	108	111	110
70	135	128	134	129	131	130
			B. Pinealectomy, Pineal-Treated Group.			
Birth	4.9	4.7	4.6	4.8	4.7	4.7
10	15.0	14.6	14.3	15.2	14.7	14.6
20	28.5	28.5	28.0	29.8	28.0	28.4
30	49.1	48.6	48.3	53.9	50.7	49.8
40	68.6	67.5	69.5	75.1	71.7	70.1
50	93.5	90.0	87.4	93.3	90.7	88.7
60	113	108	106	115	116	105
70	135	128	127	136	135	162
			C. Pinealectomy, Pineal-Implant Group.			
Birth	4.9	4.5	4.9	4.8	4.8	4.8
10	15.0	14.9	15.0	14.3	15.5	15.0
20	28.5	28.9	29.0	28.1	29.5	28.3
30	49.1	49.0	49.5	50.3	50.8	50.8
40	68.6	67.6	69.3	72.9	70.7	79.9
50	93.5	92.1	92.8	90.8	89.6	92.0
60	113	112	112	111	112	109
70	135	129	130	135	131	131
			D. Pineal Implant Group.			
Birth	4.9	4.7	4.8	4.8	4.8	4.8
10	15.0	15.6	14.9	16.0	15.0	14.8
20	28.5	26.3	28.1	30.2	28.9	27.5
30	49.1	50.2	49.8	54.0	49.6	50.2
40	68.6	70.1	69.3	72.3	71.2	71.9
50	93.5	93.0	92.3	92.3	87.5	91.2
60	113	111	112	109	113	110
70	135	131	130	134	127	135

in the opening of the ears, eruption of the teeth, appearance of hair, opening of the eyes, descent of the testes, and the opening of the vagina in the test rats when contrasted with the development observed in the operative controls.

Injection of Pineal Extract in Pinealectomized Rats. Pinealectomized rats were started on daily intraperitoneal injections of 1 cc. of aqueous HCl

pineal extract at the average age of 28 days. Six successive generations of the pineal treated pinealectomy test rats have been observed, distributed as follows: 1st generation (F_0) 6; 2nd generation (F_1) 137; 3rd generation (F_2) 122; 4th generation (F_3) 100; 5th generation (F_4) 135; 6th generation (F_5) 52, making a total of 546 animals in 71 litters of which 464 or 84 per cent survived the 70-day period of observation. The average age at which the first litter was born was 104 days, the average period between gestations 41.8 days, and the number of animals in each litter varied from 5 to 11 with an average of 7.7 rats per litter.

In table 1, *B*, are presented the average weights at 10-day intervals of the test animals in each generation. Comparison of these weights with the weights of the operative controls shows that the rate of growth of the test rats showed no definite acceleration or retardation throughout the entire 70-day period of observation. Likewise no noteworthy departure from the normal in the opening of the ears, the eruption of the teeth, the development of fur, the opening of the eyes, the descent of the testes, or the opening of the vagina was noted (table 2, *B*).

Frequent Homologous Pineal Implants in Pinealectomized Rats. Implantation was started at the age of 30 days, and continued at weekly intervals until each animal had received 12 sets of implants. The glands were obtained from stock rats varying in age from 30 to 150 days, and deliberately mixed so that the recipients received glands from both sexes of varied ages. In the parent rats of the 1st and 2nd generations, 3 pineal glands were transferred at each implantation. From the 3rd to the 5th generation, this number was increased to 5, so that by the 3rd generation, each parent had received 36 implanted glands, and from the 3rd to the 5th generation each parent had received 60 pineal glands during a similar period of implantation.

Six successive generations of pineal implant pinealectomized test rats have been observed, distributed as follows: 1st generation (F_0) 6, 2nd generation (F_1) 71; 3rd generation (F_2) 138; 4th generation (F_3) 117; 5th generation (F_4) 143; 6th generation (F_5) 135, total of 604 animals in 77 litters of which 537 or 88 per cent survived the 70-day period of observation. The average age at which the first litter was cast was 99 days, the average period between gestations was 40 days, and the number of animals in each litter varied from 4 to 11 with an average of 7.8 rats per litter.

In table 1, *C*, are presented the average weights at 10-day intervals of the test animals in each generation. Comparison of these weights with the weights of the operative controls shows that the rate of growth of the test rats showed no definite acceleration or retardation throughout the entire 70-day period of observation. Likewise no noteworthy departure from the normal in the time of opening of the ears, the eruption of the teeth, the development of fur, the opening of the eyes, the descent of the testes, or the opening of the vagina was noted (table 2, *C*).

Effects of Pineal Implants in Normal Rats. To date, 6 successive generations of the pineal implant test rats have been observed, distributed as follows: 1st generation (F_0) 6; 2nd generation (F_1) 113; 3rd generation (F_2) 164;

TABLE 2. SOMATIC DEVELOPMENT IN CONTROLS AND SUCCESSIVE GENERATIONS OF TEST RATS

	Control	F_1	F_2	F_3	F_4	F_5
A. Pinealectomy Group.						
Ears opened	2–3 (2.9)	3–4 (3.1)	3–4 (3.2)	2–4 (3.1)	3 (3)	3 (3)
Teeth erupted	7–10 (8.5)	7–11 (8.8)	6–11 (8.5)	7–10 (8.7)	7–9 (8.4)	8–9 (8.6)
Hair appeared	11–14 (12.5)	11–17 (13.6)	11–16 (9.7)	12–17 (13.8)	12–15 (13.4)	13–14 (13.6)
Eyes opened	13–17 (15.2)	13–18 (15.3)	14–17 (15.7)	14–18 (15.7)	14–17 (15.7)	15–16 (15.2)
Testes descended	24–35 (30.2)	23–35 (28)	21–30 (28.1)	22–32 (27.1)	26–32 (29.5)	27–31 (28.6)
Vagina opened	39–50 (44.3)	40–63 (45.6)	42–49 (45.1)	42–48 (45.4)	46–51 (49.1)	46–50 (48.5)
B. Pinealectomy, Pineal-Treated Group.						
Ears opened	2–3 (2.9)	3 (3)	3–4 (3–1)	3 (3)	2–3 (2.9)	3 (3)
Teeth erupted	7–10 (8.5)	7–9 (8.2)	6–10 (8.1)	8–9 (7.9)	7–10 (8.2)	8–9 (8.4)
Hair appeared	11–14 (12.5)	13–14 (13.4)	11–15 (13.1)	13–14 (13.2)	12–15 (13.2)	13–14 (13.4)
Eyes opened	13–17 (15.2)	15–16 (15.7)	13–17 (15.6)	13–16 (14.8)	14–16 (15.5)	15–16 (15.4)
Testes descended	24–35 (30.2)	26–30 (28.7)	22–38 (26.6)	22–29 (26.4)	26–31 (28.5)	25–30 (28.5)
Vagina opened	39–50 (44.3)	41–48 (45.1)	38–48 (43.9)	35–48 (44.0)	47–54 (48.7)	53–50 (48.5)
C. Pinealectomy, Pineal-Implant Group.						
Ears opened	2–3 (2.9)	2–3 (2.8)	2–4 (2.9)	3–4 (3.0)	2–3 (2.9)	3 3
Teeth erupted	7–10 (8.5)	7–9 (8.1)	5–10 (8.2)	7–10 (8.3)	6–9 (7.8)	7–9 (8.3)
Hair appeared	11–14 (12.5)	12–14 (13.0)	11–15 (13.3)	12–15 (13.6)	11–14 (12.9)	12–14 (13.3)
Eyes opened	13–17 (15.2)	13–16 (14.8)	12–16 (15.5)	10–18 (15.9)	13–16 (15.5)	13–17 (15.4)
Testes descended	24–35 (30.2)	22–31 (25.6)	21–31 (28.2)	27–30 (28.5)	24–31 (29.2)	28–33 (30.5)
Vagina opened	39–50 (44.3)	41–51 (45.9)	44–50 (45.9)	43–48 (46.3)	43–52 (47.5)	43–50 (48.1)
D. Pineal-Implant Group.						
Ears opened	2–3 (2.9)	3 (3)	3–4 (3.0)	3 (3)	3 (3)	3 (3)
Teeth erupted	7–10 (8.5)	7–10 (8.5)	8–10 (8.5)	7–10 (8.5)	6–9 (8.0)	6–9 (8.0)
Hair appeared	11–14 (12.5)	12–15 (13.4)	13–15 (13.5)	12–15 (13.7)	11–14 (12.9)	11–14 (12.2)
Eyes opened	13–17 (15.2)	15–17 (15.7)	14–17 (16.0)	14–16 (15.5)	13–17 (15.6)	14–16 (15.5)
Testes descended	24–35 (30.2)	25–34 (28.2)	22–31 (27.0)	22–31 (28.0)	26–32 (29.4)	28–32 (29.8)
Vagina opened	39–50 (44.3)	42–53 (47.0)	42–51 (46.7)	41–49 (45.3)	46–52 (48.8)	46–51 (53.2)

Figures in parentheses represent average values. All values in days.

4th generation (F_3) 134; 5th generation (F_4) 117; and 6th generation (F_5) 63, a total of 591 animals born in 74 litters of which 501 or 84 per cent survived the 70-day period of observation. The average age at which the first litter was cast was 95.5 days, and the number of animals in each litter varied from 4 to 13 with an average of 7.9 rats per litter.

Analysis of the biological data of each of these generations reveals no significant deviations from the results obtained in normal rats. In table 1, *D*, are presented the average weights at 10-day intervals of the test animals in each generation. Comparison of these weights with the weights of the control animals reveals that the rate of growth of the test rats shows no definite acceleration or retardation throughout the entire 70-day period of observation. Likewise no noteworthy departure from the normal in the time of the opening of the ears, the eruption of the teeth, the development of fur, the opening of the eyes, the descent of the testes, or the opening of the vagina has been noted (table 2, *D*).

Control Experiments with Pineal Extract in Normal Rats. In view of these consistently negative results, it seemed imperative to make certain that the pineal extract used in replacement therapy was potent in our hands when administered to normal rats. This extract was an aqueous HCl pineal extract made from desiccated defatted pineal powder from beef of killing age and prepared in this laboratory by Dr. Arthur Steinberg.[2] It differed naturally from the picric acid pineal extract (Hanson) used in our original experiments.

PINEAL EXTRACT IN SUCCESSIVE GENERATIONS OF NORMAL RATS

Three pairs of 30-day-old albino rats (Wistar strain) were selected from the normal stock animals of the Institute. These animals were mated, and started on daily intraperitoneal injections of 1 cc. of pineal extract. Data on somatic development were recorded daily, and the weights of the rats twice weekly up to the age of 70 days. All of the animals in one litter or part of a large litter of each pair of parent animals were mated, set up as parents of the next generation, and started on treatment at the average of 30 days. Thus the offspring in each generation were young born to parent rats receiving daily intraperitoneal injections of 1 cc. of pineal extract.

Effects in the First Generation (F_0). Pineal extract was administered to this group of 3 pairs of rats over a period of 8 months. The general effects consisted of a mild interference with the growth of the animals, and increased irritability.

Growth and Development of the Second Generation (F_1). A total of 142 rats was born in 18 litters to the original 3 pairs of test rats. Of this number, 114 or 80 per cent survived the 60-day period of observation. The average age at which the first litter was cast was 95 days, the interval between gestations was 40 days as compared with 42.5 days in the control series, and the average number of animals per litter was 7.9.

No definite departure from normal in the time of opening of the ears, eruption of the teeth, appearance of the hair, or the opening of the eyes was

[2] Method of preparation of this extract is described in a separate communication.

noted. However, it was observed that gonadal development was somewhat accelerated, the testes descended at an average age of 28.7 days, and vaginal opening occurred at 45.8 days. More striking than the rate of somatic differentiation was the retardation in growth shown by these rats. Although at birth, the weight of these rats was normal, further growth as shown by the average weight curve was definitely retarded, reaching a maximum of 12 per cent at 30 days. Considerable variation in the growth of individual animals of the same litter, as well as litters from the same parents was frequently observed (tables 3 and 4).

TABLE 3. AVERAGE WEIGHTS, IN GM., AT 10-DAY INTERVALS OF PINEAL-TREATED GROUP CONTRASTED WITH CONTROLS AND ORIGINAL SERIES (1)

Age, days	Controls	F_1	% Underweight	F_2	% Underweight	F_3	% Underweight	F_3 Original series
Birth	4.6	4.6	0	4.6	0	4.6	0	4
10	15.0	13.1	7	12.0	20	11.2	26	10
20	29.5	27.0	8	23.0	22	19.1	35	17
30	55.0	48.5	12	41.1	19	29.7	46	24
40	72.5	68.3	6	59.7	18	44.6	38	
50	86.0	83.4	3	77.2	10	57.9	33	
60	108	104	3	97.2	10	74.5	31	

Growth and Development of the Third Generation (F_2). A total of 142 rats was born in 19 litters of which 116 or 82 per cent survived the 60-day period of observation. The average age at which the first litter was cast was 106 days, the interval between gestations was 36 days as compared with 42.5 days in the control series, and the average number of animals per litter was 7.5.

TABLE 4. SOMATIC DEVELOPMENT OF PINEAL TREATED GROUP CONTRASTED WITH CONTROLS AND ORIGINAL SERIES (1)

	F_1	F_2	F_3	F_3 Orginal series
Ears opened	2–3 (2.9)	2–3 (2.9)	2–3 (2.3)	2–3 (2.3)
Teeth erupted	7–9 (8.0)	7–9 (7.8)	5–8 (6.2)	5–8 (6.9)
Hair appeared	11–13 (12.5)	11–13 (11.8)	9–12 (10.2)	5–12 (9.0)
Eyes opened	11–16 (14.8)	12–16 (13.9)	8–12 (10.7)	5–13 (9.8)
Testes descended	26–30 (28.7)	25–30 (27.5)	13–19 (16.0)	5–12 (10.0)
Vagina opened	46–50 (45.8)	42–48 (45.2)	33–38 (36.0)	29–39 (32.0)

Figures in parentheses represent average values.

Slight acceleration in the time of opening of the ears, eruption of the teeth, appearance of fur, opening of the eyes, descent of the testes, and opening of the vagina was noted. In 6 litters, the eyes opened between the 12th and 13th days. The rate of growth of these rats was more striking than the rate of development. At 10 days, the pineal test rats showed a retardation

in growth of 20 per cent, and at 20 days this retardation was 22 per cent. This gradually decreased so that by the age of 60 days, the pineal test rats were only 10 per cent below the weight of the control animals (tables 3 and 4).

Growth and Development of the Fourth Generation (F_3). A total of 205 rats was born in 27 litters of which 148 or 72 per cent survived the 60-day period of observation. The average interval between gestations was 27.2 days as compared with 42.5 days in the control series, and the average number of animals per litter was 7.6.

In this generation development was still further accelerated, and the rate of growth still further retarded as compared with the growth and development of the rats of the 3rd (F_2) and 2nd (F_1) generations. The ears opened in 2 to 3 days, an average of 2.3 days. The teeth erupted from the 5th to the 8th days, an average of 6.2 days. Hair appeared between the 9th and the 12th days, an average of 10.2 days. The eyes opened at 8 to 12 days, with an average of 10.7 days. The testes descended at 13 to 19 days (average 16 days) and the vagina opened at 33 to 38 days (average 36 days). The rate of growth of these rats was markedly retarded below that of the 3rd generation (F_2), 2nd generation (F_1), and control animals, reaching a maximum of 46 per cent at 30 days (tables 3 and 4).

DISCUSSION

This report is based upon investigations over a period of approximately 30 months. It covers a study of 4139 rats distributed as follows: control group 195; pinealectomy test group 669; pinealectomy split-test groups 1021; injection of pineal extract in pinealectomized rats 552; frequent homologous pineal implants in pinealectomized rats 610; pineal implants in normal rats 597; and normal rats treated with pineal extract 495.

In all of our previously reported thymus investigations, striking biological phenomena were described, i.e., accruing acceleration in the growth and development of the offspring of successive generations of treated rats was obtained with thymus excess produced by intraperitoneal injections of a potent thymus extract or by frequent homologous thymus implants, while thymus deficiency produced retardation in growth and development which could be readily overcorrected either by extract or implants. In the pineal series, significant changes in growth and development were observed only in instances of pineal excess produced by intraperitoneal injections of potent pineal extract. Pinealectomy, and replacement therapy in pinealectomized rats either with pineal extract or pineal implants gave entirely negative results. These negative results in the pinealectomy, and replacement therapy experiments cannot be explained on the basis of a change in experimental approach since the technic followed in both the pineal and thymus experiments was identical.

The behavior of the rats under treatment with pineal extract seemed abnormal; they were distinctly more irritable than controls or thymus treated animals. The mortality rate in the offspring was higher than in the thymus treated animals, reaching 28 per cent in the 4th generation (F_3). The indi-

vidual animals in the same litter varied considerably in size, and vigor. The less vigorous animals often appeared to be starved, a factor which may have contributed to the retarded average growth. At the age of 15 to 20 days, the animals presented a rather characteristic appearance, with a comparatively large round head, small body, and squatty figure.

The intervals between gestations in the 1st, 2nd, and 3rd generations was 40, 36, and 27.2 days respectively, showing a more frequent breeding in successive generations under treatment. Nine litters or 33 per cent in the 4th generation (F_3) were cast at intervals of 25 days or less. Sexually these animals were much more active than their controls.

In tables 3 and 4 are presented the data on the growth and development observed in the rats of the 4th generation (F_3) previously reported (1). Comparison of these figures shows that the rate of somatic differentiation in both groups of animals is strikingly similar. Although the rate of growth in both groups is markedly retarded, that of the original series is retarded about 50 per cent more than the series herein reported. In general, however, the data reported in the present series confirms the original findings of Rowntree, Clark, Steinberg and Hanson.

SUMMARY

Pinealectomy in successive generations of parent rats exercises no noteworthy effect in the growth or development of the offspring. The same negative results obtain when either parent alone is pinealectomized.

The daily intraperitoneal administration of 1 cc. of pineal extract or of frequent pineal implants in pinealectomized rats exercises no marked effect on the growth and development of the offspring. Frequent pineal implants in normal rats does not affect the rate of growth or development of the offspring. However, the daily intraperitoneal administration of 1 cc. of pineal extract to successive generations of rats has resulted in an increase in the frequency of breeding, an acceleration in the rate of somatic differentiation, and a retardation in the rate of growth in the offspring of treated rats. These biological effects are progressive in character becoming more marked in successive generations under treatment.

REFERENCES

1. Rowntree, L. G., J. H. Clark, A. Steinberg and A. M. Hanson: *J.A.M.A.* 106: 370. 1936; *Endocrinology* 20: 348. 1936.
2. Einhorn, N. H., and L. G. Rowntree. *Endocrinology* 21: 659. 1937; 22: 342. 1938. Einhorn, N. H., *Endocrinology*, 22: 335. 1938; 22: 435. 1938.
3. D'Amour, M. C., and F. E. D'Amour, *Proc. Soc. Exp. Biol. & Med.*, 37: 244. 1937.

SURGICAL TREATMENT OF EXPERIMENTAL INSULIN POISONING

FREDERICK M. ALLEN AND C. A. VICENS
NEW YORK CITY

As shown in a series of previously published papers (1), a sufficiently large dose of insulin can prove fatal in one of two ways: either by creating need for a fatally large quantity of glucose, or by direct toxicity of the insulin itself. The independence of this condition from hypoglycemia is established by the fact that symptoms may begin before there is a serious reduction of blood sugar and are not prevented by glucose injections which raise the blood sugar to normal or above. The symptoms consist in malaise, loss of appetite, often vomiting, reduced sensitiveness to pain, increasing weakness, stupor and collapse. Circulatory or vascular impairment is indicated by delayed absorption of injected fluids, but death always occurs by apnea while the heart continues for an appreciable time. Minor or major convulsions in the terminal stage are due more probably to injected glucose than to the insulin itself.

Hypothetically, the injury caused by excessively large or long continued glucose injections may be assumed to be either chemical or osmotic, while the direct toxicity of insulin is presumably due to an action upon the nervous system, somewhat as postulated by Sakel. The latter is the most probable explanation of the wide differences in sensitiveness of different species. Some birds are described as supremely insensitive. Rats have the highest tolerance among mammals. An average rat can tolerate 800 to 1000 U of insulin subcutaneously. A 2 to 3 kg. rabbit tolerates only about one-third this amount. A cat has about the same absolute tolerance as a rat, but with the important difference that the rat exhibits merely increased appetite and appears comfortable as long as a sufficient supply of bread is provided, while the cat loses appetite and survives with difficulty after many hours of glucose injections.

Protamine insulin is decidedly more toxic than regular insulin in both the ways mentioned. The injury through glucose is illustrated by the fact that 10 to 15 U of protamine insulin is harmless to a fed rat, but in a fasting rat it creates a need for glucose injections continued through 3 or more days, until the amount of glucose reaches such a high total as to be fatal also to control animals. The direct toxic effect is illustrated by the loss of appetite in a rat with 50 or 60 U of protamine insulin subcutaneously; nothing then avails to save the animal's life. Likewise in a cat, with a tolerance of close to 1000 U of regular insulin, death is caused by 200 to 300 U of protamine insulin. The same is generally true of rabbits, though one exceptional rabbit recovered from a dose of 250 U of protamine insulin, after continuance of convulsive reactions requiring treatment by glucose injections for one week.

Such scanty information as exists indicates that the human tolerance for insulin may be high, at least under some conditions. Certain states of abnormally high tolerance or demand for insulin are well known in diabetes, connected especially with acidosis, infections or intoxications, and certain 'resistant' states supposedly due to hyperpituitarism or other endocrine disorders. Aside from these exceptional conditions, records have been published (1) showing the administration of as much as 1000 U of insulin in an ordinary typical case of diabetes. In an unpublished case total doses of either regular or protamine insulin as high as 2000 U in 24 hours were safely borne with the aid of only moderate quantities of carbohydrate. Neither anorexia nor collapse resulted in these cases. Tests of this kind, however, must be made only with great care, particularly because of the probable wide variations of tolerance between individuals, and the serious danger of a real overdose. In the treatment of non-diabetic tuberculosis cases, a maximum of 320 U per day was taken with benefit by one man, but the great majority of such patients seem to lose appetite and become unwell with much smaller doses. These unfavorable symptoms are exhibited by occasional asthenic individuals, with or without infection, with extremely small doses of insulin. Sakel and his followers have encountered a few deaths among insane patients treated with insulin doses ranging up to several hundred units, but here the intentionally severe and prolonged hypoglycemia is so prominent that a possible factor of specific insulin toxicity cannot be accurately evaluated.

It has long been known that when animals are allowed to remain in severe hypoglycemia for too long a time, the raising of their blood sugars to any level by means of any quantity of sugar fails to save their lives, even though the dose of insulin may have been small. Attention has not been given heretofore to the equally hopeless condition which can arise from the specific toxicity of insulin in sufficiently large doses. The apparent variability of human tolerance is one point to be emphasized. As mentioned above, a few individuals develop malaise, anorexia and perhaps vomiting from very small insulin doses, and others from moderate doses. In animals, these symptoms are always danger signals; and although within certain dosage limits the saving of life is still possible with parenteral glucose injections, doses of insulin beyond these limits are fatal in spite of any known treatment. Therefore, theoretically at least, there is a possible chance that a person may take a prodigious quantity of insulin with suicidal intent, or that a person who becomes unwell with 10 to 25 U may be given many times this amount therapeutically by somebody who is unaware of the idiosyncrasy. Death may then be as inevitable as with an overdose of mercury or phosphorus, and no amount of sugar will avail as an antidote.

The question whether such a condition has ever occurred in man, or whether all deaths have been due to the primary or secondary effects of hypoglycemia, cannot be settled here. But since animals definitely die from excessive insulin doses, it is possible to perform therapeutic experiments at least from a theoretical standpoint. The present situation is that the animal is doomed from the moment of the insulin injection, even though it may

linger perhaps 1 to 3 days with the aid of glucose administration. As insulin is ordinarily given by subcutaneous, or sometimes intramuscular injection, consideration may be given to surgical measures for cutting off the supply at its source. The possible methods for stopping the supply are *a*), amputation or excision, and *b*), ligation.

AMPUTATION

Experiments with injection of insulin in the legs, followed by amputation after various periods, were previously published (1), dealing particularly with rats. Results obtained in cats are illustrated in table 1, the individual animals being selected as typical from a larger series.

TABLE 1

Cat	Insulin	Hours of Treatment			Glucose Injected, gm.			Results
		Before amputation	After amputation	Total	Before amputation	After amputation	Total	
1	1000 U regular, in leg	—	—	18	—	—	14	Lived
2	Same	—	—	26	—	—	17	Lived
3	Same, with amputation	10	23	33	9	10	19	Died in hypoglycemia
4	Same, with amputation	15	19	34	12	6	18	Died in hypoglycemia
5	1200 U regular	—	—	19	—	—	19	Died without hypoglycemia
6	Same, with excision	12	28	40	6	21	27	Died without hypoglycemia
7	320 U protamine	—	—	46	—	—	32	Died without hypoglycemia
8	Same, with amputation	20	2	22	11	1	12	Lived
9	Same, with excision	16	3	19	9	2	11	Lived

It is first noticeable that, when 1000 U of insulin was injected about the ankle and a mid-thigh amputation performed 10 or 15 hours later, cats 3 and 4 required more glucose than the controls (*1* and *2*) to prevent convulsive reactions; also the duration of treatment was considerably longer, and death occurred 23 or 19 hours after amputation (33 or 34 hours after the insulin injection), because of failure to inject still more glucose at that time. This phenomenon was explained in a former publication (1) by the fact that hypoglycemia persists in all animals for a long period, such as 24 hours, following the last active attack which requires glucose; and the shock of amputation evidently increases this hypoglycemic tendency. The main point, however, is that hyperinsulinism persisted, even in fatal degree, for as long as 34 hours from the time of injection or 23 hours after completion of absorption as established by the amputation. In other words the fatal condition here dealt with is a true constitutional hyperinsulinism which cannot be combated by amputation after as long an interval as 10 hours. Presumably considerable insulin could be removed by amputation within the first few hours after injection, but, especially because of the complicating and aggravating factor

of shock, tests of this kind were not undertaken. Incidentally, the results of shock should be noticed as adding another exception to the imagined regularity of insulin-glucose ratios.

Simple excision is probably more difficult in small mammals than in man, because of the more rapid spread of injected fluid through their looser areolar tissues, and because of the greater tendency to gravitation downward when injections are given in the legs. Trials were made with subcutaneous injections in the anterior thorax, which is dependent in the animal's natural position, followed by excision of skin and muscle over an area about 5×8 cm. The control cat, 5, receiving 1200 U died in 19 hours in spite of protection against hypoglycemia. Cat 6, in which excision was performed 12 hours after injection, lived 40 hours, requiring more glucose because of the longer survival. This difference in survival probably represents one of the accidental variations between individual animals rather than genuine benefit from the excision. Most important is the fact that the hyperinsulinism persisted for 28 hours after the excision, and that death then occurred in spite of prevention of hypoglycemia. From this it is obvious that the action of the insulin was not arrested by the operation, and the remarks concerning amputation also apply here.

It has been previously shown that protamine insulin is more toxic than regular insulin in a specific sense; also the enormously greater demand for glucose created by it adds to the disproof of the validity of insulin-glucose ratios. Thus, in contrast to the tolerance for 1000 U of regular insulin, cat 7 was killed by 320 U of protamine insulin, in spite of protection against hypoglycemia with 32 gm. of glucose during 46 hours. Cat 8, receiving the same treatment followed by amputation of the leg after 20 hours, required only one more glucose injection at the end of 2 hours; complete recovery followed. Cat 9 received the same dose subcutaneously in the thorax, and after 16 hours the injection area was excised. The injection of only 2 gm. of glucose was necessary in the 3 hours following operation, after which hypoglycemic symptoms were absent. Death ensued after 4 days, without any relation to hypoglycemia, evidently on account of a gangrenous infection in the thoracic wound, of a reddish liquefying character. Although large insulin injections leave no trace of visible injury in the undisturbed tissues, there is a question whether they lower the resistance to surgical injury, or whether this result was entirely accidental.

The main conclusion is that protamine differs radically from regular insulin, in that the prolonged effects are due chiefly to slow absorption from the site of injection, and true constitutional hyperinsulinism is only a minor feature which can be terminated rather promptly by cutting off the source of insulin supply. The time required for complete absorption in animals is much longer than heretofore suspected from human observations, amputation being effective after as long an interval as 62 hours according to experimental demonstration (1), while a still longer delay may be suspected from occasional observations of the persistence of the effects of protamine insulin up to a maximum of 7 days.

LIGATION

The use of the tourniquet for attempting to delay the absorption of poison is illustrated in the usual treatment of snake bite (2). A series of papers (3) have been devoted to a study of the periods for which tissues can endure lack of circulation without the occurrence of necrosis, also the constitutional effects in the form of shock (4). In particular, it was found that the tendency to both necrosis and shock is minimized by keeping the tissues cooled nearly to 0°C. during asphyxia.

Taking strychnine as typical of a soluble poison, and accepting 1 mg. as the lethal dose for a 2-kg. rabbit, it was learned that treatment along the lines mentioned can afford protection against a dose of 10 mg. When the injection is given in a leg, the effect of such a 10-fold dose is so rapid and powerful that it is advisable to apply a tight tourniquet within 30 seconds. After an interval of 15 to 30 minutes, the tourniquet can be released for 30 seconds. Such releases can be repeated and gradually lengthened. Local inflammation and general shock are largely prevented by keeping the leg immersed in ice-water during the periods of ligation. After some time, which may vary perhaps from 12 to 24 hours according to conditions, the periods of release may be lengthened to 15 to 30 minutes and the rabbit becomes free from symptoms.

From the above experience and from general theoretical considerations, it appears logical, if an overdose of insulin has been injected in a limb, to try a tourniquet for treatment. According to the evidence, this method of checking absorption should be promising if applied sufficiently soon after an injection of ordinary insulin, before a severe constitutional condition has developed, or at much later stages in the case of protamine insulin. Literally dozens of attempts of this kind, in rats, rabbits and cats, with ordinary and protamine insulin, ended in failure. Death has regularly occurred within shorter times than in the controls, or with far lower insulin doses than can be tolerated by the untreated controls.

These experiments are too heterogenous to be tabulated, but among the points covered are the following.

a), When a tourniquet is applied soon after the injection, symptoms remain absent until it is released. With refrigeration of the leg the animal can be kept safe and comfortable for as long as 48 hours. There is therefore no question of absorption of insulin either through a persisting circulation or by protoplasmic diffusion or other channels.

b), The fatal result is not due to temperature, for it occurs at ordinary temperatures, also when the leg is refrigerated and the body warmed artificially so as to maintain normal rectal temperatures.

c), The result also is not due to gangrene, inflammation or other local changes of any ordinary character, for these are absent or minimal especially when refrigeration is used.

d), Hypoglycemia occurs as usual but is easily overcome by glucose injections, and the animals die even if the blood sugar is kept at or above normal levels from beginning to end. Also no fatally large quantities of glucose are required for this purpose.

e), The clinical symptoms resemble those of shock, but the blood concentration characteristic of secondary shock seems to be lacking, as far as could be judged from a few blood counts subject to the unavoidable disturbing influence of the glucose injections required to prevent hypoglycemia. Also, as already mentioned (3), under ordinary circumstances the symptoms of shock are practically prevented by refrigeration during ligation, and it is reasonable to assume that the formation of histamine-like substances (5) during ligation is similarly prevented.

f), The experiments were numerous because all conceivable combinations were tried. In one type, the tourniquet was applied early and periods of release of only a few minutes were alternated with ligation periods up to several hours, in order to permit the smallest possible absorption and the longest possible intervals for consumption of the insulin. In an opposite type, the tourniquet was applied later and for shorter periods, in order to produce relatively slight lengthening of the time of absorption and correspondingly slight local tissue changes. It appeared reasonable that some combination should be discoverable, which would enable the animal to tolerate something more than the usual fatal dose of insulin, by allowing more time for the insulin to be used up in the body with the aid of sufficient glucose injections.

The fact that all such attempts only increased the fatal tendency, in a more rapid and powerful manner in proportion to the degree of interference, sufficed finally to establish one definite practical conclusion, namely that ligation is not only a failure for treating insulin intoxication, but it actually creates danger so that a tourniquet should not be applied to a limb after a very large insulin injection has been given.

On the theoretical side, certain results at one stage suggested a possible formation of a toxic substance in the asphyxiated limb under the influence of insulin. This hypothesis was overthrown by more extended observations, including control tests of the following classes. *a*), A leg was ligated as usual, but the insulin injection was given not at the beginning but at the end, that is just before or after removal of the tourniquet. *b*), At the close of the usual ligation period of one leg, the insulin was given in the opposite leg. *c*), Comparisons were made with tourniquets placed at different levels from hip to ankle, so as to expose different masses of tissue to both insulin and asphyxia, and different time limits were similarly compared. The entire explanation of the phenomena seemed to be found in a mutually aggravating influence of shock and large insulin dosage. Both the hypoglycemic and the toxic actions of insulin are increased and prolonged by shock, while the prostrating and fatal effects of shock are increased by large insulin doses and by the large glucose injections which they necessitate.

SUMMARY

Excessive insulin doses produce a state of constitutional hyperinsulinism, which may be fatal. The insulin being evidently absorbed with the usual promptness, amputation or excision of the injection site is valueless in late stages.

The prolonged effect of protamine insulin is due less to systemic insulin excess than to continued absorption from the injection site. Therefore, even at late stages, amputation or excision is an effective means of terminating the effects of an overdose of protamine insulin.

Alternate periods of ligation and release not only fail to protect animals against overdoses of either ordinary or protamine insulin, but actually increase the toxic effect. Practically, these results warn against the use of a tourniquet in any attempts to treat insulin intoxication. The effects of shock and of large insulin doses are mutually aggravating.

1031 Fifth Ave.,
New York City.

REFERENCES

1. Allen, F. M.: *New England J. Med.* 219: 77. 1938; *Tr. A. Am. Physicians* 53: 320. 1938.
2. Allen, F. M.: *South. M. J.* 31: 1248. 1938.
3. Allen, F. M.: *Tr. A. Am. Physicians* 52: 189, 1937; *Surgery* 3: 893. 1938; *Am. J. Surg.* 41: 192. 1938; *Ann. Surg.* 108: 1088. 1938; *Surg., Gynec. & Obst.* 67: 746. 1938.
4. Paper to appear in *Arch. Surg.*
5. Barsoum, G. S., and Smirk, F. H.: *Clin. Sc.* 2: 337. 1936.

THE DIABETES OF BEARDED WOMEN

(SUPRARENAL TUMOR, DIABETES, AND HIRSUTISM)
A CLINICAL CORRELATION OF THE FUNCTION OF THE SUPRARENAL CORTEX IN CARBOHYDRATE METABOLISM

H. C. SHEPARDSON AND EDWARD SHAPIRO

From the Department of Medicine, University of California Medical School

SAN FRANCISCO, CALIFORNIA

THE SUPRARENAL CORTEX is rapidly becoming an important rival of the anterior hypophysis for attention, primarily because of the unusual effects caused by disturbances in its function. Yet this widespread interest is of relatively recent origin, for, although the association between hypertrichosis, adiposity, and adrenal tumor was established as early as 1756, it was only 30 years ago that the subject was brought into prominence, especially by Broster (1) of the Charing Cross Hospital, London. More recently the suprarenogenital syndrome has been the subject of extensive investigations throughout the world. In a recent article, Broster (2) has suggested classifying the patients who present this symptom-complex (as evidenced by the appearance of secondary male sex characters in the female), into three groups: those with pseudohermaphroditism, those with virilism, and the Achard-Thiers syndrome. It is with the last division, in which the adrenal lesion seems to be but one component of a multiple glandular disturbance, that we are concerned.

Diabetes mellitus associated with tumor of the suprarenal cortex was described first by Kraus (3) in 1913. However, recent investigations into the rôle of the suprarenal cortex in the metabolism of carbohydrates attaches more than historical value to a reconsideration of the so-called *diabète des femmes à barbe*. Although the paucity of reported cases would seem to designate this condition as a medical curiosity, it is apparent that early recognition, proper diagnosis, and adequate treatment of these patients, even though infrequent, would develop information of fundamental importance in evaluating the various functions of the suprarenal cortex.

It is well known that relatively few patients afflicted with suprarenal cortical hyperplasia, or neoplasm, exhibit hyperglycemia and glycosuria. In fact, in many instances the pathologic disturbance may cause no symptoms whatsoever. Seemingly, production of clinical manifestations of disease of the adrenal cortex is dependent upon several factors chief among which is probably the type of cell involved. Langeron and Loheac (4) have classified suprarenal tumors into *a*), those producing endocrine manifestations, *b*), those presenting simply an abdominal mass with or without metastases, and *c*), those causing paroxysmal hypertension (a condition usually considered to result from tumors of the medullary portion of the gland). That type of suprarenal tumor causing masculinization of the female, a condition designated as the suprarenogenital syndrome, is alone germane to the present discussion.

The suprarenogenital syndrome has been studied with diligence by Bulloch and Sequira (5) in 1905, Apert (6) in 1910, Gallais (7) in 1912, and more recently by Broster and Vines (1),

Grollman (8), and Walters, Wilder, and Kepler (9). The clinical picture encompasses the superposition of secondary male characteristics upon fading female sex signs. Thus, masculine hypertrichosis, hypertrophy of the clitoris, changes in the larynx, and occasionally male psychologic outlook appear on the background of menstrual dysfunction, atrophy of the mammae, and changes in the bodily form. Nonsexual additions to the complex are hypertension, dusky acneic skin, striae atrophicae, obesity of the trunk, and rarely, demineralization of the skeleton or osteoporosis. The heterosexual hypertrichosis varies from a downy moustache and beard and masculine pubic escutcheon to a coarse hirsutes of the entire body. Coincident with or following the development of hirsutism occurs amenorrhea of variable degree. The contour of the body becomes less feminine as a result of the obesity, usually of the so-called 'buffalo type,' confined to the trunk and sparing the extremities. The mammary tissue atrophies, although the breasts may become larger because of the adiposity. A purplish striation of the skin over the torso and breasts is very common. The skin becomes coarse and dark; seborrhoeic dermatitis is prone to develop. The Achard-Thiers syndrome of *diabète des femmes à barbe* adds to these various abnormalities decreased carbohydrate tolerance as manifested by a diabetic type of glucose tolerance curve and glycosuria.

In reviewing the literature pertinent to this subject, difficulty is frequently encountered in determining the presence of certain essential symptoms. Indeed, in certain of the cases which might otherwise be included, the diagnosis of diabetes was inadequately established. The existence of heterosexual hypertrichosis and glycosuria in an individual does not of necessity warrant the assumption that the patient is an example of the Achard-Thiers syndrome. In some of the reported examples, uncontrolled conditions made it impossible to draw accurate conclusions concerning the metabolism of carbohydrates. And if accurate data are to be recorded in an attempt to evaluate properly the rôle abnormal suprarenal cortical function plays in the production of a clinical state in all respects resembling diabetes mellitus, it is imperative that only those patients be considered who actually present a typical symptom-complex. We have attempted, therefore, to analyze critically all of the cases reported in the literature, accepting only those in which the presence of suprarenogenitalism and diabetes is unquestioned. Each of the cases discussed has been scrutinized and studied in the original publication in order to avoid, if possible, the consideration of duplicate reports. Of a somewhat larger number recorded only 17 presented sufficient data to warrant being included as unequivocal examples of the Achard-Thiers syndrome.[1] Certain others might be acceptable except that important corroborative data were unrecorded. Thus, Guemes (10), Laignel-Lavastine and Boutel (11), Kennedy and Lister (12), Langeron and Danes (13), Vallery-Radot, Delafontaine, and Jouveau-Dubreuil (14), Walters, Wilder, and Kepler (1934, *case 1*) (8), and Goldzieher and Koster (15) have presented cases of suprarenal cortical tumor with transitory glycosuria, but the diagnosis of diabetes was uncertain. Aschner (16), Labbe and Boulin (17) and Weil, Weissmann-Netter, and Renaudeaux (18) have recorded cases simulating the Achard-Thiers syndrome; however, the evidence of a suprarenal lesion was lacking, the diagnosis being deduced by exclusion and inference.

Appended hereto is a brief abstract of each of the accepted cases. In addition, in table 1 is shown the percentile distribution of the salient diagnostic characteristics of the Achard-Thiers syndrome in this group. Diabetes, hirsutism, hypertension, and amenorrhoea are the outstanding clinical phenomena in this symptom-complex. It is impossible to explain the menorrhagia in Tuffier's (19) patient and the normal catamenia in the patient reported by us, unless the former case was that of an arrhenoblastoma. (See second case summary below.)

Obesity, acneiform dermatitis, striae, and enlargement of the clitoris are variable and may be related to the stage of the disease, being present

[1] Two of these are doubtful. They are included for historical reasons. The diagnosis of diabetes in the case Kraus (3) recorded is extremely uncertain. Likewise the diagnosis of diabetes in the patient reported by Achard and Thiers (34) (from whom the symptom complex derives its name) is questionable.

TABLE 1. THE MAJOR SIGNS AND SYMPTOMS IN 18 CASES OF "DIABETES OF BEARDED WOMEN"

	cases	per cent
Diabetes	18	100
Hirsutism	18	100
Hypertension	17	94
Amenorrhea[1]	15	83
Obesity	13	72
Dusky, acneic skin	9	50
Terminal loss of weight	5	28
Striae atrophicae	4	22
Enlarged clitoris	3	17

[1] Included as amenorrhea are the cases of Achard—71-year-old post-menopausal patient; Oppenheimer—12-year-old pre-pubertal patient; Brown—patient who had had a hysterectomy previously; Shallow—patient who had had a hysterectomy previously; Duncan—56-year-old post-menopausal patient.

more frequently in cases of long duration. Terminal loss of weight is almost uniformly associated with malignant cortical lesions. In the series reported, over half of the cases presented evidence of a malignant neoplasm (see table 2). It has been claimed that polycythemia is partially responsible for the florid facies; in the 12 cases in which blood counts were studied, only 4

TABLE 2. THE HISTOLOGIC TYPE OF SUPRARENAL CORTICAL LESION IN 18 CASES OF "DIABETES OF BEARDED WOMEN"

	cases	per cent
Carcinoma (proved)	7	39
Carcinoma (clinically)[1]	2	11
Hyperplasia and/or adenoma	7	39
Type not noted	2	11

[1] 'Carcinoma, clinically' indicates that although neither post-mortem nor biopsy was obtained, a typical syndrome was present with evidence of metastases.

showed suggestive or certain evidence or erythrocytosis. Persistent alkalosis occurred in the patients studied by Walters, Wilder, and Kepler (9) (*case 1*) and by McQuarrie, Johnson, and Ziegler (20); this is inexplicable. The diverse basal metabolic rates of 12 of the patients in this series (presented in table 3) are explainable on the basis of a thyrotoxicosis which was present in

TABLE 3. THE BASAL METABOLIC RATES RECORDED IN 10 CASES OF "DIABETES OF BEARDED WOMEN"

Case	B.M.R., %	Comment
5	−3 to +1	No autopsy
20	+2.2	Normal thyroid at autopsy
14	+4	Atrophic thyroid at autopsy
15	+13	Patient still living
8	+20	Colloid adenomatous hyperplasia of thyroid at autopsy
13	+20	Recurrent multiple colloid and fetal adenomata with interadenomatous parenchymatous hypertrophy of the thyroid, grade 2.
6	+27	Normal thyroid at autopsy
4	+15 to +28	Thyrotoxicosis clinically for 2 years; storage type of goiter at autopsy
16	+34	Normal thyroid at autopsy
12	+26 to +50	Toxic adenoma of the thyroid for 20 years; patient still living

several of the patients before or coincident with disease of the suprarenal cortex. Such data should not be used to corroborate the thesis that the Achard-Thiers syndrome is a polyglandular disease, or that the associated hyperglycemia was incident to the thyrotoxicosis.

In the main, the patients considered to be examples of the Achard-Thiers syndrome presented the signs and symptoms of diabetes mellitus superimposed on the background of suprarenogenital disease. Of the latter there seems to be no pathognomonic diagnostic symptomatology.

We have had the opportunity of observing a fairly typical example of this rather rare condition. A somewhat detailed report of this case follows.

CASE REPORT

S.R. A single white American female box-maker, aged 30 years, entered the University of California Hospital February 5, 1935, and died March 11, 1935. On entry her chief complaints were hypertrichosis of the face, of 13-year duration and acne of the face and shoulders, of 4-year duration. Since puberty the patient had noted increased hairiness of the body and thighs. When she was 17 years of age hair had begun to appear on the chin, cheeks, and upper lip to an extent requiring increasingly frequent depilation. She had been slightly overweight (160 lb.), but in the preceding four years had lost 30 lb. During this same interval she had developed an acneiform rash of the face and shoulders. For 4 months preceding entry, polyuria and polydipsia had been troublesome. For 3 months she had suffered from headaches, and during this same interval pain unrelated to food intake or bowel habits had developed in the left flank. Since its onset, menstruation had been irregular, occurring from every 3 to 5 weeks. It is of extreme interest, however, that for the 3 months preceding entry her menstrual periods had occurred regularly at each 28-day interval. Furthermore, there had been no alteration in the amount or duration of flow.

The patient's father had had diabetes and had died at 58 years of age of 'heart disease.' Her mother had died at 51 years of age of 'apoplexy.' The patient had had measles, mumps, and chicken pox in early childhood. She also had suffered an attack of scarlet fever at the age of 12 years, and rheumatic fever at the age of 13 years, both without recognized sequelae. At the age of 23 years she was stricken with pyelonephritis, with occasional mild recurrences.

The patient was a woman of masculine appearance with thick stubbly black hirsutes on the chin, cheeks, and upper lip (fig. 1, 2 and 3). Coarse hair was present all over the body; the pubic escutcheon was of the male type. The skin was oily and acneic. Adiposity was moderate, and of the buffalo type. Her weight on entry was 62.8 kg. (138 lb.).

The body measurements were: height 155.5 cm.; (62¼ in.), span, 163.0 cm.; (65¼ in.), crown-symphysis, 77 cm.; symphysis-floor, 78 cm.; intertrochanteric, 95 cm.; breast circumference, 88 cm.; and waist circumference, 81 cm.

The eyes were large and rather prominent. The palpebral fissures measured 12 mm. right and 11 mm. left. The exophthalmometer readings with the bar at 100 were 22 mm. right and 21.2 mm. left. The right pupil was smaller than the left and did not react to light. The extraocular movements, visual fields, and fundi were normal. The lymph nodes were not palpable. The ears, nose and throat were normal. The thyroid gland was normal in size and consistency. The breasts were pendulous and large. The thorax was normal in appearance; percussion developed a normal resonance and auscultation showed no significant findings. The area of cardiac dulness

was not enlarged, the heart sounds were regular; no murmurs were heard. The arteries were not unduly sclerotic. The pulse was regular and of good quality. The blood pressure varied from 140/100 to 190/110. Palpation of the abdomen demonstrated no masses. Pelvic examination showed a virginal outlet with intact hymen and no hypertrophied clitoris; by rectal palpation the uterus and cervix were found to be infantile. The extremities were normal. The deep reflexes were equal and active; no abnormal reflexes could be elicited.

Fig. 1. Photograph of S.R., showing the hirsutes and seborrhic skin.

Laboratory Findings

Blood: Hgb. 12.8 gm. (88%). R.B.C. 4,350,000. W.B.C. 10,800. Differential count—P.M.N. 64%, P.M.E. 1%, P.M.B. 1%, S.L. 31%, Mono. 3%. Platelets—190,000.
Bleeding and Clotting Times: normal.
Blood Sugar (fasting): 268 mg. %.
Carbon Dioxide Combining Power: 49.1 vol. %.
Glucose Tolerance Curve: (mg. %): 268, 344, 533, 513.
Plasma Cholesterol: 236 mg. %.
Whole Blood Chlorides: 429 mg. %.
Non Protein Nitrogen: 33.3 mg. %.
Urine: Pale, yellow, cloudy, acid, sp. gr. 1022. Albumin, heavy trace. Acetone, negative. Glucose, complete reduction of Benedict's solution. Sediment: (centrifuged) loaded with leucocytes and erythrocytes. Occasional granular casts. Bacilli present.
Phenolsulfonphthalein Renal Function Test (during ureteral catheterization): normal.
Urine Culture: Bacillus coli.
Basal Metabolic Rate: 2.2+%.
Blood and Spinal Fluid Wassermann Tests: Negative in all dilutions.
Colloidal Gold Curve (spinal fluid): normal.
Roentgenograms of skull, chest, spine: Negative.
Retrograde pyelograms were interpreted by the genitourinary department as showing slight distortion of the left renal pelvis, suggestive of tumor. However, the roentgenologist reported no evidence of suprarenal tumor.

On a diet consisting of CHO 140 gm., P 70 gm., and F 90 gm. (cal. 1670), with 40 to 65 u of insulin daily, at least one of the specimens in the routine 4-period test

gave an 'orange' reduction. Because of continued loss of weight, the diet was increased to CHO 160 gm., P 80 gm., and F 110 gm. (cal. 1950). This regime demanded from 65 to 100 U of insulin daily, and again at least one of the urine specimens showed marked glycosuria but without acetonuria (fig. 4).

In spite of treatment with urinary antiseptics (and for a time a ketogenic diet), the polyuria and bacilluria persisted. Nevertheless, the patient was afebrile after the first week.

Fig. 2. S.R., showing the 'buffalo' obesity and bodily hypertrichosis.

Because of the difficulty of controlling the diabetes, and considering the associated hirsutism, the questionable roentgenogram of an enlarged adrenal, and in the hope of favorably influencing carbohydrate metabolism, bilateral suprarenal exploration was performed on March 7, 1935. A total of 15.3 gm. of adrenal tissue was removed, 11.5 gm. being taken from the left. (It will be recalled that the clinical findings suggested a left suprarenal tumor.) The blood pressure fell to 112/90 after the operation; hence in addition to parenteral glucose and Ringer's solution the following drugs were administered during the first 24 hours: 1 cc. of eschatin intravenously and 1 cc. of 1:1000 epinephrin hydrochloride subcutaneously every hour; 0.025 gm. of ephedrin sulfate every 2 hours. The blood pressure rose to 180/100 at the beginning of treatment but soon dropped, so that during the first 3 post-

operative days the systolic pressure ranged from 84 to 135 mm. On the fourth day after operation, the blood pressure was 84/60. By this time the temperature of the patient had slowly risen to 41.2° C. In spite of renewed treatment with eschatin and epinephrin the patient died at 7:35 A.M., March 11, 1935.

Post-mortem examination revealed the following pertinent findings. The remaining suprarenal cortical tissue consisted of 21 gm. on the right and 7 gm. on the left. The surgical specimens showed hyperplasia with small adenomatous nodules in the cortex and both remnants showed a similar hyperplasia; in the right gland an adenoma 1.5 cm. in diameter was discovered in the pole opposite the resected portion. The pancreas, although small, was normal histologically. The thyroid, thymus and pineal glands were normal; over 90 serial sections through the pituitary, which

UNIVERSITY OF CALIFORNIA

UNIVERSITY HOSPITAL — OUTPATIENT DEPARTMENT

DIABETIC CHART

Name S.R. (Pre-operative Course) Number 81,743

Age 30

DATE 1935	DIET C.	P.	F.	CAL.	INSULIN (units)	WEIGHT (lbs.)	URINE 1	2	3	4	URINE ACETONE	BLOOD SUGAR	BLOOD CHOLES-TEROL	PLASMA CO2 (vol.-%)	OTHER DATA
2-5	Regular					68.8			Gr	Gr	0				BMR 8.2% +
2-7	Regular					61.8	Gr	Gr	Gr	Gr	0	268		49.1	G.T. curve
2-8	140	70	90	1670	0-0-40		Gr	Yel	Bl	Gr	0				268, 344,
2-15					35-0-20	59.7	Bl	Bl	Bl	Bl	0				333, 313.
2-18					40-5-20	59.6	Gr	Gr	Gr	Gr	0				Pl. Chl. 554
2-20	160	80	110	1950	40-5-20	59.2	Cl	Gr	Gr	Gr	0				Wh.Bl.Cl.429
2-23					40-5-20	59.9	Gr	Gr	Gr	Bl	C				Cystoscopy
2-26					55-10-25-10		Cl	Bl	Gr	Gr	0	187#			
	Ketogenic diet for urinary infection from 2/27 to 3/4														
3-2	60	40	180	2020	30-5-15	58.0	Gr	Bl	Bl	Bl	+			56.9	
3-4	150	80	110	1950	25-10-15		Gr	Bl	Bl	Bl	+				
3-6					35-10-20		Gr	Gr	Gr	Bl	0				
	Operation at 9 a.m. on March 7, 1935														

Fig. 3. Preoperative diabetic chart of S.R., demonstrating the difficulty in controlling the diabetes.

weighed 0.75 gm. failed to reveal the presence of an adenoma. The ovaries weighed 6 gm.; microscopically the ovarian tissue appeared somewhat atrophic, and no active formation of graafian follicles was evident. The uterus was normal. Also noted were an acute cystitis and bilateral pyelonephritis with abscess formation, and terminal bronchopneumonia.

DISCUSSION

It immediately becomes apparent that confusing conditions existed. There is no doubt that the suprarenogenital syndrome was present, nor can there be any question of an associated diabetes. That a suprarenal tumor, in the form of an adenoma in the cortex of the right adrenal as well as a bilateral cortical hyperplasia, were causative factors seems almost beyond peradventure of a doubt. Nevertheless, several abnormalities usually considered to be of the utmost diagnostic significance were entirely lacking in the patient. No diminution in the menstrual function was apparent. On the contrary, the periods became more regular and the character and volume of the flow improved during the three months preceding entry into the hospital. The enlargement of the clitoris was questionable, for, in retrospect, the impression is obtained that there was only a slight hypertrophy which was within

UNIVERSITY OF CALIFORNIA

UNIVERSITY HOSPITAL OUTPATIENT DEPARTMENT

DIABETIC COMA CHART

Name S.R. (Post-operative Course) Number 81,743

Age 30

TIME		URINE			BLOOD SUGAR	INSULIN	FLUIDS		BLOOD CO.	BLOOD CHOLEST.	NOURISHMENT				REMARKS
DATE	HOUR	AMOUNT	ACETONE	DIACETIC	MGM. PCT.	(units)	PARENT	MOUTH	VOL. PCT.	MGM.	CHO	P	F	CAL.	
3-7	7 am	31	0	0		30	750 cc. 10% glucose i.v.								
	8:30						1000 cc. Ringer's sol.								To Surgery
	12						1000 cc. Ringer's sol.								To Ward
	4 pm					30	750 cc. 10% glucose i.v.								
	7	Cr	0								ESCHATIN			1	cc.
	10:30					30	500 cc. 10% glucose i.v.								
	12	Cl	0				total 4000 cc.				ESCHATIN			1	cc.
3-8	6 am	Cl	0								75	40	40	820	
	10						1500 cc. Ringer's sol.								
	12					20					ESCHATIN 1 cc. hourly from 12:30 a.m. until 6:30 a.m.				
	2	Cl	0												
	4:20					20	750 cc. Ringer's sol.								
	6	Cl	0												
	8	Cr	0												
	10	Cl				15	total 2515 cc.								
3-9	2 am	Cl	0								75	40	40	820	
	9	Cr	0			25	-								
	11	Cr	0			20	1500 cc. Ringer's sol.								
	4 pm	Cr	0			25									
	10	Cr	0				total 2320 cc.								
3-10	7 am	Cl	0								140	60	90	1540	
	6	Cr	+												
	7					25									
	9	Cl	+												
	11	Cl	+												
	12					20									
	1 pm	Cr	+			20	1000 cc. 10% glucose i.v.								
	2:20	B	+												
	3:20	B	+												
	4	B	+			25									
	5	B	+		410	25	2500 cc. Ringer's sol.								
	6	B	+			25					ESCHATIN			1	cc.
	6:45	Cr	0			20									
	7	Tr	0			15					ESCHATIN			1	cc.
	7:45	Cr	+			20									WBC - 11,000
	8:15	Cl	+			15					ESCHATIN			1	cc.
	8:45					20									
	9	Cl	+			25					ESCHATIN			1	cc.
	9:30					25									
	10	Cr	0			20									
	10:30	Cr	0			20					ESCHATIN			1	cc.
	11	Cr	0			20									
	11:30	Cr	0								ESCHATIN			1	cc.
	12					15	2500 cc. Ringer's sol.				ESCHATIN			1	cc.
3-11	1 am	Cr	0			20									
	1:30	Cr	0								ESCHATIN			1	cc.
	2					15									
	2:30	Cr	0								ESCHATIN			1	cc.
	3					15									
	3:30	Cr	0			15					ESCHATIN			1	cc.
	4:30	Cr	0								ESCHATIN			1	cc.
	5:30	Bl	0												
	6:30	Bl	0								ESCHATIN			1	cc.

Fig. 4. Post-operative diabetic chart of S.R., showing the insulin and eschatin therapy.

the limits of normal variation. Osteoporosis, considered by many to be of significant importance, was not shown on repeated radiological examinations of the bones. Polycythemia was absent.

It is interesting to note the difficulty experienced in properly controlling

the diabetes. Although this patient undoubtedly had a severe disturbance in carbohydrate metabolism, it was our impression that her insulin requirement was much too great for the degree of diabetes that seemingly was present. It is possible, that, quite like other instances of diabetes associated with profound alterations in function of almost any of the members of the hormonopoetic system, this is a typical example of insulin refractory diabetes, not so much because of any failure of insulinogenic activity of the pancreas, but rather because of the marked disturbance in metabolic processes resulting from definite changes in incretory function of, in this instance, the suprarenal cortex.

Finally, it should be mentioned that even had this patient survived the bilateral partial suprarenalectomy and had not succumbed to bronchopneumonia, as well as to cortical insufficiency, a cure of the Achard-Thiers syndrome probably would not have supervened. The exploration of the right adrenal gland, and in fact removal of a portion for microscopic study, failed to reveal the presence of a large adenoma which is presumed to be the primary causative factor in the production of the entire symptom-complex. Only during examination at necropsy was the tumor discovered. This represents what may prove to be an insurmountable technical difficulty in making an adequate diagnosis from biopsy alone.

The Mechanism of Diabetes in the Achard-Thiers Syndrome. Since Blum (21) at the turn of the century, first noted that epinephrin injection caused glycosuria in normal animals, the relation of the suprarenal gland to carbohydrate metabolism has been the source of much study. Early experiments involving ablation of both the suprarenals and the pancreas were doomed to insignificance since the animals died of cortical insufficiency. However, the preparation of potent extracts of the suprarenal cortex, as a result of the work of Rogoff and Stewart (22), Swingle and Pfiffner (23), and Hartman (24) has permitted research on suprarenalectomized animals over an extended period.

Long and Lukens (25) have shown that bilateral extirpation of the suprarenal glands in animals causes *a*), imperfect absorption of dextrose, *b*), hypoglycemia on fasting, *c*), sensitivity to insulin, *d*), resistance to the hyperglycemic action of epinephrin, *e*), rapid depletion of liver glycogen by fasting, and *f*), failure of muscle glycogen resynthesis after exercise. Although sensitivity to insulin may be partially due to the coincident removal of the suprarenal medulla, these manifestations resemble greatly the disturbances in carbohydrate metabolism resulting from hypophysectomy. It will be recalled that Houssay demonstrated that the diabetes produced by pancreatectomy is partially relieved by removal of the anterior hypophysis, in that there is an increased ability to oxidize dextrose (26) and an inhibition in the capacity to form dextrose from non-carbohydrate substances (27), with a consequent reduction of hyperglycemia, glycosuria, and ketosis. Similarly adrenalectomy ameliorates experimental pancreatic diabetes, although, as shown by Long (28), the survival period of adrenalectomized-pancreatectomized animals, while almost three times longer than the lifespan of the control, pancre-

atectomized animals, was considerably shorter than the survival period of hypophysectomized-pancreatectomized preparations. Yet it seems probable that the mechanism of relief in the adrenalectomized animals is identical with that obtained in Houssay's animals.

Furthermore, the fact that hypophysectomy induces atrophy of the suprarenal cortex (29), indicates that the action of the anterior hypophysis on carbohydrate metabolism may be by way of the suprarenal cortex. As Long (28) suggests, "it would be expected that just as thyroidectomy abolished the effect of the thyrotropic hormone, adrenalectomy would render ineffective any influence of the hypophyseal hormones upon metabolic processes."

Many observers (30) have noted that hyperglycemia and glycosuria follow the injection of extracts of the anterior hypophysis into normal dogs. Furthermore, hypophysectomized-pancreatectomized dogs can be thrust easily into uncontrolled diabetes and ketosis by small amounts of these extracts. Long (28) demonstrated that suprarenalectomy prevents the diabetogenic function of anterior pituitary extracts, thereby adding further evidence that the effect of the adenohypophysis on carbohydrate metabolism is via the suprarenal cortex. The long awaited corollary was reported recently by Lukens and Dohan (31); large doses of suprarenal cortical hormone reestablished the diabetes of suprarenalectomized-pancreatectomized animals.

Finally, it is well known that patients with Addison's disease (32), as well as animals with experimental destruction of the suprarenal cortex (33), show hypotension, hypoglycemia, and sensitivity to small doses of insulin. And the postulate that a suprarenal tumor producing an excess of cortical hormone results in phenomena diametrically opposite to those found in Addison's disease has been shown to be reliable by McQuarrie, Johnson, and Ziegler (20).

Clinically, then, it may be expected that in patients with the Achard-Thiers syndrome the diabetes will be markedly ameliorated by eliminating the production of excess cortical hormone. This has actually been accomplished by Walters, Wilder, and Kepler (9). Resection of bilateral hyperplastic suprarenal cortices in a woman of 31 years of age (*case 15*) caused a return to normal of the hypertension and hyperglycemia, and the heterosexual hirsutism disappeared. The menstrual status subsequent to operation was not mentioned.

As yet, however, conclusive evidence that the adrenal cortex is solely responsible for the entire symptom complex is lacking. Possibly no condition illustrates as completely as does the Achard-Thiers syndrome the interrelationship of the hormonopoietic system. Rapidly accumulating evidence seems to indicate conclusively that proper function of many of the organs of internal secretion is dependent on the normal activity of other members of the system, just as any function of the body is controlled by several interrelated agencies which are mutually dependent and highly sensitive to changes in any other.

The exact mechanism of the production of diabetes by excess or abnormal

suprarenal cortical hormone, in spite of rather extensive investigations into the relationship of the adrenal cortex to carbohydrate metabolism, is as yet a moot question. It seems probable, however, that the anlage of diabetes must be present in those patients who develop the Achard-Thiers symptom-complex. In no other way can explanation be made of the fact that diabetes occurs only occasionally in association with the suprarenogenital syndrome. Yet evidence at present available seems to indicate that suprarenal cortical hyperfunction is an etiologic factor in the production of the Achard-Thiers syndrome. Furthermore, it seemingly successfully refutes the remark (15) that this dyndrome "comprises cases of apparently heterogenous origin similar only in respect to disturbed carbohydrate metabolism and masculine secondary sex characteristics." Yet one must conclude after critical analysis of all the pertinent facts that the abnormal suprarenal function is but one element (although probably the most important) of a multiple glandular disturbance involving not only the suprarenal cortex, but the incretory portion of the pancreas, the ovaries and possibly the adenohypophysis as well.

SUMMARY

Although the relationship between virilism and adrenal cortical tumor was suggested as early as 1756, and the association of diabetes with tumor of the suprarenal cortex was first described in 1913, the entire symptom-complex, the so-called *diabète des femmes à barbe* was correlated by Achard and Thiers in 1921. A careful scrutiny of the literature has brought to light only 17 cases which may be included as unequivocal examples of this syndrome. A brief abstract of each of the accepted cases is appended. The eighteenth such case, a fairly typical example of this rather unusual condition, is reported.

The theories pertinent to the production of diabetes in the Achard-Thiers syndrome are discussed. It becomes evident, however, that the suprarenal cortex is not solely responsible for the disturbance in carbohydrate metabolism. There seems to be little doubt that alterations in function of the suprarenal cortex profoundly affect the insulinogenic activity of the pancreas. However, diabetes occurs only occasionally in association with the suprarenogenital syndrome. One is forced to the conclusion, therefore, that in those patients who develop the Achard-Thiers syndrome, the anlage of diabetes must be present.

Critical analysis of relevant data indicates that in the Achard-Thiers syndrome suprarenal function is but a single, although probably morbific element of a multiple glandular disturbance involving in addition the hormonogenic portion of the pancreas, the ovaries, and possibly the adenohypophysis.

APPENDIX

Case 1. Kraus (3) 1913. A woman, aged 25 years, had been ill with albuminuria for 3 years. Her menses were normal since onset at the age of 13, until 2 years previously when amenorrhea supervened. She grew no more after her 13th year, and although formerly quite obese, she had lost 30 lb. in the past few months. Since the menarche she had presented heterosexual hirsutism over the entire body, with a prominent beard. The genitalia were normal. Glycosuria and hypertension were present. The clinical diagnosis was hypernephroma.

Case 2. Tuffier (19) 1914.[2] A woman, aged 62 years, presented herself with the complaint of metrorrhagia. She exhibited marked heterosexual hirsutism, with a prominent beard and moustache which had developed after the menopause. The clitoris measured 4 cm. The blood pressure was 200/130. Glycosuria to 70 gm. per day was reduced to as low as 6 gm. per day by dietary regulation. The sella was roentgenographically normal. Operation for the supposed leiomyoma uteri revealed palpable masses 'half the size of the kidney' in both suprarenal regions. Also noted were endometrial hyperplasia, atrophy of the left ovary, and a tumor 'the size of a walnut' on the right ovary; microscopic examination of the ovarian tumor was inconclusive. The glycosuria disappeared 3 months after the laparotomy.

Case 3. Achard and Thiers (34) 1921.[3] A woman, aged 71 years, presented a masculine facies with heavy moustache and beard, and generalized heterosexual hypertrichosis; she stated that the beard had begun to grow when she was 9 years old. The menses had started at the age of 12 years and had persisted until she was 60. The clitoris was normal. Urinalysis at the age of 69 revealed glycosuria of 'several' grams, easily corrected by dieting. During her hospitalization, glycosuria was never noted, and could not be produced either by the ingestion of 100 gm. of dextrose or by the injection of epinephrin. The blood pressure was 200/100. She died 16 days later in a very cachectic state. At autopsy, the right suprarenal gland weighed 9 gm., the left 7 gm., and both showed increased pigmentation and hyperplasia. The pancreas presented pericanalicular fibrosis. Also demonstrated were: early portal cirrhosis, chronic thyroiditis, and sclerosis of the ovaries.

Case 4. Keyser and Walters (25) 1924. A woman, aged 37 years, had noted amenorrhea and obesity for 3 years, enlargement of the thyroid with thyrotoxicosis for 2 years, oiliness and pustulation of the skin for 1½ years, hirsutes of the face, arms, and chest, with thinning of the hair of the scalp for 1 year, and coarseness of the voice for 1 year. Examination showed a masculine habitus, adenoma of the thyroid, blood pressure ranging from 124/90 to 180/110, and a smooth abdominal mass palpable below the left costal margin. The urine contained a moderate amount of albumin and from 5.9 gm. to 6.5 gm. of glucose per 24-hour specimen. The fasting blood sugar varied from 0.111 to 0.153 per cent. The blood urea was increased to from 34 to 38 mg. per cent. The B.M.R. ranged from +15 per cent to +28 per cent. The sella turcica was roentgenographically normal. At operation a large tumor of the left suprarenal was removed; histologically, it was found to be a carcinoma. She died 4 days later. Autopsy showed a normal pituitary gland and normal ovaries. The follicles of the thyroid gland were lined with flattened epithelium and were distended with colloid. Hemorrhagic fat necrosis in the pancreas also was noted.

Case 5. Oppenheimer and Fishberg (36) 1924.[4] A girl aged 12 years had been noted to gain about 75 lb. (34 kg.) in one year beginning when she was 5½ years of age. One year before admission the skin had become florid and hair had grown on the face, axillae, and pubis. She had never menstruated. Urinalysis at this time revealed 4 per cent sugar. Examination showed an undersized obese child with red face and marked hairiness. Abscesses were present on the back and neck and there were ulcers on the legs. The abdomen was marked with pigmented striae. The heart was enlarged; the blood pressure was 190/130. Urinalysis on admission showed glycosuria, albuminuria, but no acetonuria; the sediment was normal. Renal function was normal. The fasting blood sugar was 0.260 per cent. The B.M.R. was normal. On an 'antidiabetic diet' and digitalis, the urine became free of sugar and albumin, and the skin infections healed. She died 3 weeks after leaving the hospital; autopsy was not obtained.

Case 6. Strauss (37) 1928. A woman, aged 41 years, had had facial hirsutism necessitating shaving since puberty; later hairiness of the limbs occurred. The menses which began at the age of 16 years had always been very irregular, and had ceased entirely for the preceding 3 months. For 6 months she had suffered from headaches, and had exhibited albuminuria. She was somewhat obese, with a red flushed face, and heavy moustache and beard, although the hair on the scalp was thin. There was slight exophthalmos. The heart was enlarged to the left, the blood pressure was 175/100, and the ankles were edematous. The abdomen was distended by an enlarged uterus. Urinalysis showed 1.4 per cent glucose without acetone; the glycosuria was easily controlled by a dietary regime. The fasting blood sugar ranged from 0.083 per cent to 0.144 per cent. The glucose tolerance test with 50 gm. of grape sugar showed a peak of 0.216 per cent in 1 hour and a level of 0.146 per cent at 3 hours. The B.M.R. was +27 per cent. Cystoscopy revealed a fibrinous cystitis. The patient aborted spontaneously and died shortly thereafter of cardiac failure. At autopsy, the left suprarenal gland was larger than the right, and presented a hard encapsulated tumor, described histologically as being malignant. The thyroid gland was somewhat hard, but otherwise normal.

Case 7. Winkel (38) 1928. A woman, aged 36 years, presented obesity for 11 years, amenorrhea for 10 years, and headaches and heterosexual hirsutism for 1 year. Examination showed her face to be

---

[2] The menorrhagia, endometrial hyperplasia, and undiagnosed ovarian tumor offer the possibility of an alternate diagnosis of arrhenoblastoma. This case is included mainly for historical interest.

[3] Certainly there is doubt that the glycosuria in this case represented a true diabetes. This case also is included because of its historical interest.

[4] This patient may have had Cushing's syndrome, since there was no proved suprarenal lesion.

florid and to bear a heavy moustache and beard. There was mild exophthalmos, but the thyroid was normal in size. The clitoris was the size of a 'hazel-nut.' The right thorax contained a pleural transudate and there were other signs of cardiac decompensation. The blood pressure was 225/145. No abdominal mass was palpable. Urinalysis showed albuminuria and glycosuria, but no acetonuria; the sediment was normal. The fasting blood sugar ranged from 0.123 per cent to 0.144 per cent. Glycosuria varying from 4 gm. to 6 gm. daily, bore no definite relation to carbohydrate ingestion, and was refractory to insulin therapy. The glucose tolerance curve was consistent with mild diabetes, reaching a peak of 0.350 per cent. Polycythemia was present. Roentgenographic examination showed the left kidney to be 'two finger-breadths higher than the right'; there was no oval shadow at the upper pole of the latter. The sella was normal. At operation, a tumor of the right adrenal was found; examination showed it to be a hypernephroma. The patient died 2 days post-operatively; post-mortem examination showed atrophy of the thyroid, suprarenals, ovaries, uterus, and vagina. An interstitial nephritis was noted. The pancreas and pituitary were normal.

Case 8. Brown (39) 1928. A woman, aged 45 years, complained of polydipsia, anorexia, weakness and numbness of the legs for 6 weeks following a hysterectomy for menorrhagia. The face was florid and pigmented with purpuric spots. The eyes were slightly prominent and there was a slight tremor of the hands. The blood pressure was 240/160. Obesity was marked and was of the 'buffalo type.' The urine showed albumin, pus, blood and 7.2 per cent sugar with a trace of diacetic acid. The fasting blood sugar was 0.370 per cent rising to 0.413 per cent 1 hour after the ingestion of 50 gm. of glucose. The blood sugar level vacillated greatly and was reflected in the varying insulin requirement. The B.M.R. was +20 per cent. The blood urea was 31 mg. per cent. There was slight polycythemia. Roentgenograms showed no enlargement of the sella turcica. The patient became drowsier, complained of cramps in the legs, and developed striking hirsutes of the chin and upper lip coincident with frontal baldness. Five months later she died of nephritis and heart failure. Autopsy revealed bilateral suprarenal hyperplasia, colloid adenomatous thyroid hyperplasia, enlargement of the pituitary gland with much colloid in large vesicles of the intermediate lobe, normal pancreas, enlarged fibrotic ovaries, and 'one typical oat-celled carcinoma 1 cm. in diameter' in the lungs. The thymus showed fatty degeneration and atrophy.

Case 9. Hunter, McMillan, Boyd and Cameron (40) 1931. A woman, aged 30 years, even as a small girl had had slight hairiness of the face. Thyroidectomy had been done several years before the present illness. In the preceding 2 years, heterosexual hirsutism had progressively increased; during this period her menses ceased, and she complained of severe backache unrelieved by the usual methods, and of increasing weakness. In spite of a good appetite she decreased in weight from 180 to 130 lb. Examination showed a florid face, a heavy moustache and beard, 'buffalo' obesity, acneic skin streaked with striae atrophicae, and heterosexual hypertrichosis of the entire body. The blood pressure was 185/120. A nontender smooth mass 'larger than a fetal head' was palpable in the right hypochondrium. The clitoris was enlarged and 'curiously hard.' The vagina was filled with a polypoid mass protruding through the cervix. There was marked albuminuria, constant glycosuria, but no abnormal constituents in the urinary sediment. The urine was kept sugar free on a diet of 85 gm. of carbohydrate, 60 gm. of protein, 140 gm. of fat plus 8 U of insulin. The glucose tolerance curve was that of moderately severe diabetes with a peak of 0.370 per cent. Renal function was markedly impaired as tested by the urea clearance, phenolsulfonphthalein excretion, and concentration tests. Polycythemia had been noted 2 years before, but the present examination showed a mild hypochromic anemia. The B.M.R. was +4 per cent. Roentgenograms showed a metastatic 'cannon-ball' lesion in the right lower chest. The sella was normal. Decalcification of the lumbar vertebrae was noted. Sections of the tumor removed from the vagina were interpreted as suprarenal carcinoma.

Case 10. Broster, Vines, Hill and Greenfield (41) 1932. A girl, aged 18 years, had noted the sudden development of amenorrhea, obesity (gain of 35 lb.), heterosexual hirsutism, and headaches 2 years previously. Examination showed obesity, limited mainly to the trunk and breasts, pink striae over the abdomen and thighs, dryness and floridity of the skin, and hairiness of the face, back and shoulders; the pubic escutcheon however, remained feminine. The blood pressure was 145/90. The urine occasionally showed traces of sugar. A glucose tolerance test with a maximum blood sugar of 0.220 per cent was indicative of mild diabetes. Exploratory laparotomy showed an enlarged right suprarenal gland, which histologically was 'not much different from the normal.' The lack of improvement following right suprarenalectomy was attributed to the probable bilaterality of the lesion. Two years later the amenorrhea, obesity, and hypertension were still present; a glucose tolerance test showed a further decrease in carbohydrate tolerance. However, the headaches and hirsutes were greatly improved.

Case 11. Shallow (42) 1933. A woman, aged 27 years, had noted obesity of the face and increased hairiness of the body for 1 year, weakness for 7 months, and polyphagia for 2 months. Metrorrhagia and irregular menstruation had been so prominent that she 'was even operated on' for the bleeding years before. The systolic blood pressure varied from 140 to 220 mm. The external genitalia were normal. There was no glycosuria, but the glucose tolerance test showed a peak of 0.278 per cent at 2½ hours. Roentgenograms revealed a large left kidney; the sella turcica was normal. At operation a suprarenal

tumor was found, examination of which demonstrated it to be an 'adenoma.' She did not show the expected improvement and 5 months later was still obese. Urinalysis at this time revealed glycosuria with an excretion of from 0.3 per cent to 0.9 per cent dextrose. Roentgenograms disclosed pulmonary metastases.

Case 12. Duncan and Fetter (43) 1934.[5] A woman, aged 56 years, had noted for 20 years a generalized hypertrichosis, masculine habitus, nervousness, and goiter. Headaches, dyspnoea on exertion, and edema of the ankles were complained of for 4 years. Three days prior to admission she noted the onset of vertigo and a tendency to fall forward; several days before these symptoms appeared, glycosuria had been discovered. Examination showed a masculine-appearing obese woman with marked facial and bodily hirsutism. The thyroid gland was enlarged and nodular. The heart was slightly enlarged, there was moderate generalized arteriosclerosis; the blood pressure was 180/110. The genitalia were normal. Glycosuria and albuminuria were noted. The fasting blood sugar was 0.173 per cent. The B.M.R. ranged from +26 to +50 per cent. There was slight polycythemia. Renal function was normal. Retrograde pyelograms suggested the presence of a mass occupying the major portion of the upper pole of the left kidney. The sella turcica and skull were roentgenographically normal. The clinical diagnosis was suprarenal cortical tumor, hyperthyroidism, and diabetes mellitus. The patient refused thyroidectomy, and in spite of roentgen therapy to the thyroid and reduction in weight, she required a diet of 240 gm. of carbohydrate, 80 gm. of protein, 180 gm. of fat (2900 calories) plus 36 U of insulin to maintain her weight and prevent glycosuria.

Case 13. Walters, Wilder, and Kepler (9) 1934. A woman, aged 46 years, had had a thyroidectomy when she was 28 years of age with recurrent thyrotoxicosis necessitating a second operation 10 years later. She always had had an insignificant growth of facial hair, but in the preceding 3 months hirsutism had become marked enough to require shaving. For 2 months she had become increasingly weaker and noted amenorrhea. In spite of a good appetite, she decreased in weight from 240 to 208 lb. (108.8 to 94.3 kg.). The face was florid and hairy, the skin warm and moist, and there was a slight manual tremor; the thyroid gland could not be felt. The blood pressure was 176/80. The left retina showed a small amount of hemorrhage and exudate; visual fields were normal. Glycosuria was constant and intense; the fasting blood sugar was 0.289 per cent. Renal function was normal. The B.M.R. was +20 per cent. Roentgenograms revealed a normal sella turcica and slight osteoporosis; intravenous urograms were normal. Spontaneous persistent alkalosis with hypochloremia and low blood volume were, inexplicably, present. A course of roentgen therapy to the pituitary gave some temporary subjective improvement. She died 2 months after admission. At autopsy hyperplasia of both suprarenal glands was found. Also noted were a small thymic tumor, a small abscess of the pancreas, intra-adenomatous parenchymatous hypertrophy of the thyroid (grade 2).

Case 14. Walters, Wilder, and Kepler (9) 1934. A woman, aged 30 years, noted increasing weakness, hairiness and floridity of the face, loss of weight, amenorrhea, and edema of the legs for 2 months. The musculature of the extremities was weak and flabby almost to the point of paralysis, a condition believed to be due more to a metabolic fault than to a neuropathy. The liver was moderately enlarged and pitting edema was present up to the level of the lower thoracic vertebrae. The blood pressure was 118/70. There was slight albuminuria, marked glycosuria, and a fasting blood sugar of 0.323 per cent. The spinal fluid was normal. Roentgenograms showed slight osteoporosis; intravenous urograms were normal; the sella was not examined. The B.M.R. was +4 per cent. At operation, a tumor of the left suprarenal gland was removed, and interpreted microscopically as carcinomatous. The pathologist suggested that it resembled a hepatoma. Post-operatively the blood sugar concentration oscillated widely; the daily requirement of insulin decreased from 65 to 10 U. The patient died 6 week slater. Autopsy revealed a carcinoma of the head of the pancreas with metastases to the peritoneum and nearby lymph nodes. The liver was almost entirely replaced by the cancer. The right suprarenal gland appeared exactly like the operative specimen. Neither the pituitary nor thyroid glands were examined.

Case 15. Walters, Wilder, and Kepler (9, 44) 1934. A woman, aged 31 years, had complained o asthenia and nervousness for 2 months, and for 2 days generalized edema which had first appeared in the face; at this time the blood pressure was 150/80, and the urine contained a slight amount of albumin and a few erythrocytes. One month later her blood pressure had risen to 180/90, a fine papular rash had appeared on the body, and glycosuria was intense. On a weighed diet plus insulin she had regained her strength and had lost much of the edema. Two months later she became irritable, forgetful, and repeated phrases. Her weight had decreased from 130 to 105 lb. (59 to 47.6 kg.). Examination showed her face to be swollen and round, with a growth of fine hair on the upper lip. The back and chest were covered with comedones and pustules. The clitoris was not enlarged. Roentgenographically the skull was normal. The fasting blood sugar was 0.154 per cent and the urine was free of sugar on a diet of 108 gm. of carbohydrate, without insulin.

---

[5] The subsequent course of this patient is not recorded.

Five months later, the acne had disappeared, the mentality was improved, and the blood pressure had fallen to 116.76. She complained of weakness of the legs, however, and had not menstruated for 3 months. Two weeks after this improvement, the acne and facial swelling reappeared, and in 3 months polyuria, polyphagia, peculiar mental reaction, and return of the menses occurred. One month later (14 months after the onset of the illness) the blood pressure was 158/96, the urine contained 4.56 per cent sugar, and the fasting blood sugar was 0.234 per cent. The CO_2 combining power was persistently elevated: 68.8 to 87.5 volumes per cent. The B.M.R. was +13 per cent. The values of sodium, potassium, and the chlorides were reduced. On a diet of 111 gm. of carbohydrate, 65 gm. of protein, and 152 gm. of fat, 40 to 45 U of insulin daily were needed to prevent glycosuria.

At operation, marked enlargement of both suprarenal glands was found, microscopically described as suprarenal cortical hyperplasia. Portions of both glands were removed. Post-operatively, the blood pressure returned to the normal value of 124/62, and the glycosuria disappeared even on a diet of 250 gm. of carbohydrate. The values for the CO_2 combining power, chlorides and potassium became normal.

For 2 months post-operatively, the hirsutes of the face had entirely disappeared, the skin was clear, the blood pressure was 120/80, and there was no glycosuria on an unrestricted carbohydrate diet.

Case 16. Bottin (45) 1936. A woman, aged 26 years, had noted amenorrhea for 4 months, facial and bodily heterosexual hirsutism for 1 month, and polyphagia and polydipsia. Eight days before admission she had spat up a small amount of blood. Examination showed a small woman with obesity limited to the trunk and abdomen. The skin was acneic and the face and body were covered with long hairs. The heart was enlarged; the blood pressure was 190/130. In the right hypochondrium was felt an irregular 'fist-sized' mass. The clitoris was hypertrophied; the breasts were normal. Glycosuria ranged from 2.7 per cent to 15.3 per cent. The fasting blood sugar varied from 0.140 per cent to 0.150 per cent. Renal function was normal. The plasma chlorides measured 0.320 per cent. The B.M.R. was +34 per cent. Roentgenographic examination showed an irregular shadow causing some compression of the right kidney. Small metastatic nodules were demonstrated at the bases of the lungs. The sella turcica and intestinal tract were normal. Biopsy of the right suprarenal tumor proved the mass above the right kidney to be carcinomatous. Roentgen therapy was begun, but the patient died shortly thereafter. At autopsy the right suprarenal was replaced by a tumor weighing 900 gm.; metastases were noted in the lungs and liver. The ovaries were sclerotic. The pancreas and thyroid were normal.

Case 17. McQuarrie, Johnson, and Ziegler (20) 1937. A woman, aged 44 years, had presented obesity and heterosexual hypertrichosis for one year. For 10 months she had noted polydipsia, nycturia, weakness and headache. Amenorrhea had been present for 7 months. Examination showed a 'buffalo type' of obesity, hairiness of the face, purplish striae over the abdomen, thighs and breasts, and moderate exophthalmos. The thyroid gland was not palpable. The blood pressure varied from 140/92 to 210/130. There were no abdominal masses. Urinalysis showed faint albuminuria, moderate glycosuria, but no diacetic acid. The renal function was normal. The fasting blood sugar was 0.387 per cent. The plasma CO_2 combining power was 112 volumes per cent; the PH of the blood ranged from 7.55 to 7.60. Roentgenograms showed a normal sella turcica and also no distortion of the kidneys. The patient died 10 weeks after entry. At autopsy the suprarenal glands were moderately enlarged, weighing 12.4 gm. and 14 gm. each. Neither adenoma nor hyperplasia were definitely identified microscopically. The pituitary gland, thyroid, pancreas, and ovaries were normal. The authors consider that the enlargement of the suprarenals indicated previous hyperplasia.

REFERENCES

1. Broster, L. R., and Vines: The Adrenal Cortex. Lewis. 1933.
2. Broster, L. R.: *Arch. Surg.* 34: 761. 1937.
3. Kraus: *Deutsch. med. Wchnschr.* 39: 2377. 1913.
4. Langeron, L., and P. Loheac: *Ann. de méd.* 24: 249. 1928.
5. Bulloch, W., and J. H. Sequira: *Tr. Path. Soc. London*, 66: 189. 1905.
6. Apart, E.: *Bull. soc. de pediat. de Paris* 12: 501. 1910.
7. Gallais, A.: Le syndrome génito-surrénal (étude anatomo-clinique), Thèse de Paris. 1912.
8. Grollman, A.: The Adrenals, Williams and Wilkins. 1936.
9. Walters, W., R. M. Wilder, and E. J. Kepler: *Ann. Surg.* 100: 670. 1934.
10. Guemes, A.: *Semana med.* 34: 207. 1917.
11. Laignel-Levastine, and A. Boutel: *Bull. et mém. Soc. méd. d. hôp. de Paris.* 44: 1303. 1920.
12. Kennedy, C. M., and W. A. Lister: *Lancet* 213: 749. 1927.
13. Langeron, L., and A. Danes: *Paris méd.* 73: 145. 1929.
14. Vallery-Radot, P., P. Delafontaine and Jouveau-Dubreuil: *Bull. et mém. Soc. méd. d. hôp. de Paris* 49: 74. 1933.
15. Goldzieher, M., and J. Koster: *Am. J. Surg.* 27: 93. 1935.
16. Aschner, B.: *Z. f. klin. Med.* 112: 43. 1929.
17. Labbe, M., and Boulin: *Bull. et mém. Soc. méd. d. hôp de Paris* 48: 1097. 1924.
18. Weil, M. P., R. Weissmann-Netter and M. Renaudeaux: *Gaz. méd. de France* 15: 373. 1930.

19. Tuffier, M.: *Bull. Acad. de méd.* 71: 726. 1914.
20. McQuarrie, I., R. M. Johnson and M. R. Ziegler: *Endocrinology* 21: 762. 1937.
21. Blum, F.: *Deutsche Arch. f. klin. Med.* 71: 146. 1901.
22. Rogoff, J. M., and G. N. Stewart: *Science* 66: 327. 1927.
23. Swingle, W. W., and J. J. Pfiffner: *Anat. Rec.* 44: 225. 1929 (abstract).
24. Hartman, F. A., C. G. McArthur and W. E. Hartman: *Proc. Soc. Exper. Biol. & Med.* 25: 69. 1927.
25. Long, C. N. H., and F. D. W. Lukens: *J. Exper. Med.* 63: 465. 1936.
26. Houssay, B. A.: *New England J. Med.* 214: 961. 1936.
27. Houssay, B. A.: *New England. J. Med.* 214: 963. 1936.
28. Long, C. N. H.: The influence of the pituitary and adrenal glands upon pancreatic diabetes, Harvey Lecture, 1936–1937.
29. Smith, P. E.: *Am. J. Anat.* 45: 205. 1930.
30. John, O'Mulvenny, Potts and Laughton, H. M. Evans, Baumann and Marine, Houssay, Lucke, E. I. Evans, and Barnes and Regan, quoted by Collip, J. B.: *J.A.M.A.* 104: 827. 1935.
31. Lukens, F. D. W., and D. C. Dohan: *Endocrinology* 22: 51. 1938.
32. Loeb, R. F., D. W. Atchley, E. M. Benedict and J. H. Throsher: *J. Exper. Med.* 57: 775. 1933.
33. Harrop, G. A., L. J. Soffer and J. H. Trescher: *J.A.M.A.* 100: 1850. 1933.
34. Achard, S., and J. Thiers: *Bull. Acad. de méd.* 86: 51. 1921.
35. Keyser, L. D., and W. Walters: *J.A.M.A.* 82: 87. 1924.
36. Oppenheimer, B. S., and A. M. Fisbherg: *Arch. Int. Med.* 34: 631. 1924.
37. Strauss, H.: *Klin. Wchnschr.* 7: 27. 1928.
38. Winkel, M.: *Deutsche Arch. f. klin. Med.* 159: 1. 1928.
39. Brown, W. H.: *Lancet* 2: 1022. 1928.
40. Hunter, C., J. C. McMillan, W. Boyd and A. T. Cameron: *Canad. M. A. J.*, 25: 188. 1931.
41. Broster, L. R., H. G. Hill and J. G. Greenfield: *Brit. J. Surg.* 19: 557. 1932.
42. Shallow, T. A.: *Ann. Surg.* 98: 297. 1933.
43. Duncan, G. G., and F. Fetter: *M. Clin. North America* 18: 261. 1934.
44. Walters, W., R. M. Wilder and E. J. Kepler: *Proc. Staff Meet. Mayo Clin.* 9: 400. 1934. *Proc. Staff Meet. Mayo Clin.* 9: 661. 1934.
45. Bottin, J.: *Liege méd.* no. 9. 1936.

THE EFFECT OF SALTS ON THE DIABETES INSIPIDUS FOLLOWING POSTHYPOPHYSECTOMY IN THE RAT[1]

H. G. SWANN AND B. J. PENNER

From the Department of Physiology, University of Chicago

CHICAGO, ILLINOIS

THE PARTICIPATION of the pars nervosa of the hypophysis in water metabolism has been abundantly demonstrated in various animals (3). Its ablation or its degeneration sequent to denervation is followed by a pronounced polyuria and polydipsia. Since the antidiuretic hormone that it secretes, probably pituitrin, is generally thought to act by controlling renal tubular absorption (4), and since this absorptive process is intimately connected with salt metabolism, it is not surprising that salt has been found to affect these experimental diabetes. Oehme and Oehme (5) reported that sodium chloride exerted a polyuric action on diabetes insipidus, and this has been experimentally verified by Bailey and Bremer (6), Curtis (7), Fisher, Ingram and Ransom (3), and Bellows (8). In this study, the effects not only of NaCl but also of other common salts on experimental diabetes insipidus are reported.

METHODS

Each rat was kept in an individual metabolism cage with a wire cloth bottom, the cage being set on a funnel so that the urine could be easily collected for daily measurement. Drinking solutions were kept in open beakers and the amount drunk measured daily with correction for evaporation. The measurements of urine output in the rat are less accurate than those for intake since the small volumes of urine are much reduced by evaporation, absorption on the funnel, and so on. Consequently although both intake and output of fluids were measured daily, only the figures for intake will be presented in this paper. The diet employed was bread and milk fresh daily, 'Calf Meal' (Cooperative G. L. F. Mills) ad libitum, and meat and lettuce scraps twice weekly. The food was given in dishes set into the side of the cage in such a way that food could not be pulled out and dropped into the urine.

The posterior hypophysis, including pars nervosa and pars intermedia, was removed by the method of Richter (9) and Pencharz, Hopper and Rynearson (10). This operation has been designated a 'posthypophysectomy.' By this technic only the thin plate of anterior lobe tissue lying ventral to the posterior lobe is removed, leaving intact the main bulk of the anterior lobe lying lateral to each side of the posterior lobe. At autopsy, serial sections of the infundibulum and the contents of the sella turcica were made for

[1] Preliminary reports (1, 2) have been published.

histologic examination. Control rats were usually subjected to a sham operation, that is, exposure of the pituitary without removing any portion of it.

The salts were administered to the rats in their drinking solutions. Equinormal concentrations were employed, usually 0.154 normal. The test solution was given for 3 weeks or more. Preceding and following the tests, control periods were made during which the rat's intake of tap water was measured.

RESULTS

In table 1 are shown the solutions tested, the number of rats, and the daily intake, averaged for a week, of operated and control groups, before, during, and after the test solution. NaCl, in all concentrations tested, aggravated the diabetes insipidus considerably. In the very high concentration of 0.308N (1.8 per cent), it was lethal to 6 out of 7 posthypophysectomized rats in about a week. These animals at the start consumed rapidly increasing quantities of the saline. In a few days they evidently reached a limit, for collapse and death soon followed. Normal rats can apparently handle this drinking solution without embarrassment, for during the 3 weeks

TABLE 1. EFFECT OF SALTS ON THE FLUID INTAKE OF POSTHYPOPHYSECTIOMIZED RATS

No. rats	Test solution	Concentration in N	Number of cc. of solution consumed per day (weekly average) during administration of				
			Water 1st wk.	Test solution 2nd wk.	Test solution 3rd wk.	Test solution 4th wk.	Water 5th wk.
12 operated	NaCl	0.068	16	29	35	33	15
4 controls	NaCl	0.068	10	13	12	16	6
8 operated	NaCl	0.154	13	36	33—	41	11
3 controls	NaCl	0.154	8	15	14	13	8
1 operated	NaCl	0.308	14	60	106	116	—
2 controls	NaCl	0.308	10	12	10	13	10
4 operated	$NaHCO_3$	0.154	19	28	30	31	20
2 controls	$NaHCO_3$	0.154	7	10	9	9	8
4 operated	Na citrate	0.154	20	22	29	21	18
2 controls	Na citrate	0.154	12	13	21	14	12
4 operated	Na_2SO_4	0.154	22	23	19	22	18
2 controls	Na_2SO_4	0.154	3	7	6	7	5
6 operated	KCl	0.154	27	22	21	24	16
3 controls	KCl	0.154	10	12	10	8	11
4 operated	KCl	0.308	21	26	31	30	26
2 controls	KCl	0.308	4	7	5	6	8
4 operated	$CaCl_2$	0.154	17	10	8	7	16
2 controls	$CaCl_2$	0.154	15	11	11	8	19
4 operated	Mammalian Ringer's		13	36	34	28	17
2 controls	Mammalian Ringer's		7	17	18	13	5

they were given this solution, their good health was attested by the fact that their average gain in weight was 16 gm.

The record of the single posthypophysectomized rat which did not die within a week when given 0.308N NaCl as drinking solution is shown in greater detail in figure 1. The slow increase in fluid intake, concurrent with a drop in weight, reached extreme limits just before the drinking solution was changed for water. At this point the animal was near death. For 3 weeks this rat drank daily more than its own weight in fluid—the most extreme protracted diabetes insipidus noted in this series or to be found, as far as we can ascertain, in the literature. The rat also set a record for a single day's

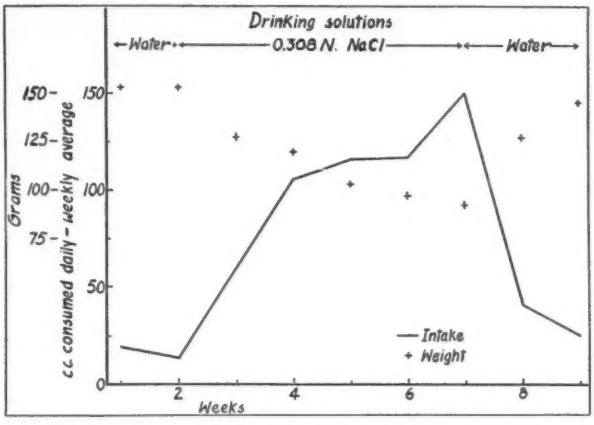


Fig. 1. Fluid intake and weights of posthypophysectomized rat drinking 0.308N NaCl.

intake: in one day it consumed 170 cc. when at a weight of 100 gm.—a polydipsia of 1700 cc. per kilo of body weight.

Recovery from the aggravation of the diabetes insipidus caused by saline solutions was usually instantaneous, that is, during the first 24 hours after the saline solution was changed for water, the rat's intakes dropped to the same level as that observed before the saline administration. This generality holds for all of this series with two exceptions. The rat discussed in the paragraph above took 3 days to recover. Also, rats which had been kept very diabetic with saline for a long time did not recover when given water to drink, but continued mildly diabetic.

Mammalian Ringer's solution and $NaHCO_3$ caused, as table 1 shows, aggravations of the diabetes insipidus similar to those caused by NaCl. But the other sodium salts tried, including Na citrate and Na_2SO_4, exerted no diabetic effects. A few observations, not here included, have also shown that di-sodium and mono-sodium phosphate solutions were similarly ineffective in making the rats more diabetic.

The other salts tested, including KCl, in two concentrations, and $CaCl_2$, were also negative (table 1); evidently posthypophysectomized rats handle these two salts normally.

In table 1 it will be noticed that each concentration of sodium chloride tends to cause gradual increases in the fluid intake. If the animals were kept on the saline drinking solutions over very long periods, these increases continued. Figure 2 shows a protocol typical of a group of 4 rats given only

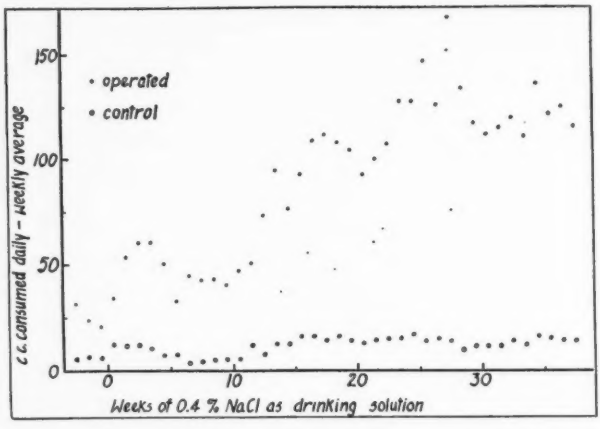


Fig. 2. Fluid intake of posthypophysectomized rat drinking 0.068N (0.4%) NaCl.

0.068N (0.4 per cent) NaCl as drinking water over a period of 38 weeks. The increase in intake was very slow, taking 15 weeks before it showed clearly. In spite of this long-continued fluid exchange (about two-thirds of the body weight daily), the rats remained in good health, with weights constant or increasing and regular estrous cycles.

It has been shown previously (2) that, after posthypophysectomy in the rat, the diabetes insipidus slowly becomes reduced, until after about 2 months the rat's fluid exchange is within the normal range. On autopsy, these rats were found to have considerable amounts of 'pars neuralis' tissue; in the terminology of Fisher, Ingram and Ranson (3), this refers to the tissue of similar appearance, and therefore presumably of similar function, to be found in the pars nervosa, infundibulum and hypothalamus. The Ranson group have suggested that incomplete removal of all this tissue is responsible for the transient diabetes insipidus seen in the dog after clipping the stalk. With regard to our recovered rats, it was reasoned that in the operation all the posterior hypophysis must have been removed except a few fragments around the base of the stalk, and that these furnished a start for the regenerating pars nervosa. After a long period, this process is completed, both anatomically and functionally, and therefore the rats are no longer diabetic. It was also reasoned that the nervosa was not left behind at operation since the pars intermedia had been all removed and since the main bulk of the pars nervosa clings in the operative technic to the intermedia.

That this recovery from diabetes insipidus can be prevented by saline administration is clearly shown; the rats get worse rather than better (fig. 1,

etc.). On autopsy of these unrecovered saline treated rats, we expected to find some alteration in the process of pars nervosa regeneration. However, histologic examination of the contents of the sella turcica and the stalk failed to reveal any clear difference between their nervosa tissue and that seen in recovered rats.

Pituitrin will prevent these saline diureses. Figure 3 shows the results of injecting various doses of pituitrin at various times to 3 rats averaging intakes of about 60 cc. daily of 0.154N NaCl. Injections spaced close together were more effective than those spaced far apart. It was also noted that the improvement caused by the pituitrin was long-lasting. The daily intake of the 3 rats, averaged for each of the 3 succeeding weeks following the cessation of injections was 23, 30 and 37 cc. In substance, the improvement caused by the pituitrin had not yet worn off after 3 weeks.

To determine whether NaCl solutions cause any diuretic effects in totally hypophysectomized rats, 5 such animals were given 0.154N NaCl as drinking solution a week after operation. Their average daily intake, taken over 1-week periods for the 3 weeks during which they were drinking the saline, was 14, 12, and 6 cc. The normal rat on this same solution drinks at about the same rate (table 1). Similarly 0.308N NaCl produced little or no diuresis in the totally hypophysectomized rat.

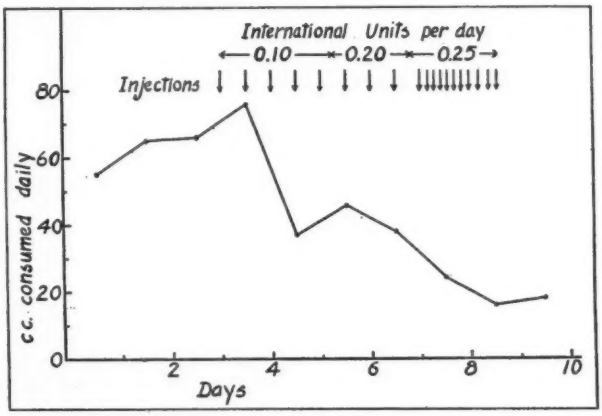


Fig. 3. Effect of pituitrin on the diabetes insipidus caused by saline solution.

It was also found that NaCl solutions would not produce any diuresis in rats which had been posthypophysectomized a long period before. A group of 7 rats, posthypophysectomized from 100 to 480 days before, without any subsequent treatment, were tested. During one week, the group averaged an intake of 11 cc. per day of tap water; during the 2nd, 3rd and 4th week they averaged, respectively, 14 cc., 14 cc. and 13 cc. per day of 0.154N NaCl. Evidently no diuresis beyond that of normal rats was caused in these rats by this saline solution. Similarly, even 0.308N NaCl caused no diuresis in rats that had been long posthypophysectomized.

DISCUSSION

It is evident that the posthypophysectomized rat handles NaCl solutions in a manner far different from the normal. Since $NaHCO_3$ produces the same sort of diabetes, it once seemed that the sodium ion was responsible for the effect. But since neither Na citrate or Na_2SO_4 exerts this effect, it appears that the sodium ion alone is not the cause. Similarly the chloride ion alone is not responsible because its presence in combination with potassium or calcium does not aggravate a pre-existing diabetes insipidus. The best that can be said about these salt effects is that they are neither a general electrolyte effect nor even a sodium ion or chloride ion effect but rather they are specific sodium chloride and sodium bicarbonate effects.

The diabetes insipidus of these salt-treated rats reaches comparatively great heights. Postoperative diabetes in the rat seldom reaches levels of fluid intake equal to 500 cc. per kilogram per day. The same holds for dogs and cats. But under the salt treatment this figure has been, in our experience, so common as not to merit particular notice. More uncommon are conditions under which rats consume the relatively enormous amount of fluid of 1000 cc. per kilo per day—or quantities equal in weight to the animal's own body weight. Even this is rather easily demonstrated by withholding fluid from a moderately diabetic rat for 24 hours and then giving it saline ad libitum; such treatment leads to a very severe diabetes insipidus for a day or so. The most extreme diabetes that we have seen—that of the rat given 1.8 per cent saline as drinking water—has been discussed above.

The saline aggravation of these operative diabetes in the rat is not apparently prevented by the regenerating pars nervosa tissue. In order to account for this and certain other phenomena, we have set up the working hypothesis that the saline diabetes of these posthypophysectomized rats are different in kind from the diabetes caused by posthypophysectomy. Applying this theory, the reason the posthypophysectomized animal given saline is so much more diabetic than after posthypophysectomy alone is that the resulting diabetes is the sum of two different diabetes—one due to posthypophysectomy and a second due to saline. Recovery from the diuresis of posthypophysectomy occurs fairly soon but the saline diabetes, once initiated, continues unchecked. Although a polydipsia is probably not the cause of posthypophysectomy diabetes, it may, as Bellows (8) suggests, play a large rôle in saline diabetes. It may also be that the three stages of diabetes insipidus, seen in the cat and dog after denervation of the pars nervosa, represent different stages in the two types of diabetes: the transient phase would be equal to the true posthypophysectomized diuresis seen in our rats; the latent phase would represent recovery from this; and the permanent phase would be equivalent to our saline diabetes in that NaCl is its main cause. This NaCl presumably comes from the animal's food—significantly, fasting the diuretic cat at once reduces its fluid exchange (11). We have observed the same effect in the rat after posthypophysectomy, and Richter (12) has shown that the degree of diabetes insipidus is directly proportional to the food intake. Similarly, Bailey and Bremer (6) found that fevers and illness

prevent an existing diabetes insipidus. The 'general metabolic level' that Richter (12) and Fisher, Ingram and Ranson (3) postulate would, in terms of our theory, be nothing more mysterious than the amount of NaCl that the animal ingests in its food. If it ingests none, as in fevers or fasting, then there is no salt intake and therefore no diabetes.

In another paper (13) it will be shown that a thyroidectomy interferes only slightly if at all with an existing saline-aggravated diabetes insipidus, even though the operation caused the oxygen consumption to decrease 44 per cent. Evidently, a depressed basal metabolism does not prevent diabetes insipidus of this special type.

It should be emphasized that the diabetes insipidus of a given animal is unquestionably not a simple affair and that the separation we have arbitrarily made of the condition into two types can only be made theoretically at the present moment. Actually, the diabetes insipidus of most animals is a combination of the two types to a greater or less degree. This consideration, further confirmation of and various difficulties in this working hypothesis will be discussed in subsequent papers.

SUMMARY

NaCl, given in the drinking solutions, causes extreme aggravation of the diabetes insipidus following removal of the posterior hypophysis in rats. In concentrated solutions, it may even cause the animal's death. It also prevents the spontaneous recovery from the operative diabetes insipidus seen in the rat. Pituitrin prevents these saline aggravations. Mammalian Ringer's solution and $NaHCO_3$ are similarly diuretic, but Na_2SO_4, Na citrate, KCl and $CaCl_2$ exert no diuretic effects on the diabetic rats beyond their effects on the normal rat.

A working hypothesis is presented that NaCl plays an important rôle in the diabetes insipidus following posthypophysectomy.

REFERENCES

1. Swann, H. G., and B. J. Penner: *Am. J. Physiol.* 123: 199. 1938.
2. Swann, H. G.: *Proc. Soc. Exper. Biol. & Med.* (In Press.) 1938.
3. Fisher, C., W. R. Ingram and S. W. Ransom: Diabetes Insipidus, etc., Edwards, Ann Arbor, 1938.
4. Burgess, W. W., A. M. Harvey and E. H. Marshall: *J. Pharmacol. Exper. Therap.* 49: 237. 1933.
5. Oehme, C., and H. Oehme: *Deutsches Arch. f. klin. Med.* 127: 261. 1918.
6. Bailey, P., and F. Bremer: *Arch. Int. Med.* 28: 773. 1921.
7. Curtis, G. M.: *Arch. Int. Med.* 34: 801. 1924.
8. Bellows, R. T.: *Am. J. Physiol.* 123: 14. 1938.
9. Richter, C. P.: *Am. J. Physiol.* 110: 439. 1934.
10. Pencharz, R. T., J. Hopper and E. H. Rynearson: *Proc. Soc. Exper. Biol. & Med.* 34: 14. 1936.
11. Fisher, C., W. R. Ingram and S. W. Ranson: *Arch. Neurol. & Psychiat.* 34: 124. 1935.
12. Richter, C. P. The Pituitary Gland, Williams & Wilkins, Baltimore, 1938.
13. Swann, H. G., and P. E. Johnson: *Endocrinology* (In press).

BUILD IN RELATION TO MENSTRUAL DISORDERS AND OBESITY[1,2]

LEONA M. BAYER

From the Departments of Medicine, Gynecology, and Pediatrics of the Stanford University School of Medicine

SAN FRANCISCO, CALIFORNIA

THE SEARCH for relationships between human configuration and human disease have intrigued physicians since the beginning of medical history. Such relationships are always elusive, especially when symptoms so common as obesity and menstrual disorders are referred to a variable so complex as body build. Nevertheless, demonstration of at least a limited relationship between symptoms and physique has been possible in this paper.

A first step in the study was the choice of standards of build. The constants established on school children by Gray and Ayres (1) were used for girls up to 19 years of age, and those for working women by Bayer and Gray (2) were used for the women of 20 years of age and older. The standards for working women are especially appropriate, since they were established for the present purpose, from the measurements of women having the same socio-economic status as the patients reported on in this paper.

Another preliminary step was the demarcation of the syndromes included in the analysis. Because normal weight and normal menstruation are indicators of a harmonious constitution in a healthy woman, upsets in either or both may occur in association with almost every kind of pathology. somatic disease, or disturbed psychic states, or states of endocrine imbalance may alike be responsible for abnormal cycles or abnormal weight. Even states of endocrine imbalance may be of two kinds: those characterized by a monoglandular syndrome, in which weight or menstrual disorder is incidental—as in gigantism, dwarfism, or acromegaly; and those obscurer states in which the obesity or disturbed menstruation is dominant. Only patients presenting the latter, obscurer states, have been examined here, because they suggested a working hypothesis. It is this: such patients often show an upset in the field of sexual differentiation or so-called maturation. In these patients, the functional dyscrasia, at least if it originates before adolescence, should be reflected in the body build.

MATERIAL AND METHODS

Since 1930, anthropometric and clinical studies have been made on 110 women and girls whose complaints were thought possibly to be associated

[1] Aided by the Rockefeller Fluid Research Fund.

[2] Collection of data was made possible by the interest of Drs. H. Gray, C. F. Fluhmann and H. K. Faber, who helped in the study of suitable cases in their respective clinics.

with their build. In this study, those with obesity or menstrual disorders or both have been sifted, and 90 cases are finally considered. Their distribution according to dominating symptom and age is shown in table 1.

TABLE 1. DOMINATING SYMPTOM

	Children ages 6–19	Adult ages 20–57	Total
Menstrual disorder	12	34	46
Obesity	12	24	36
Thyroid disorder	1	5	6
Ectodermal dystrophy	0	2	2
Total	25	65	90

The racial background of the series is essentially Northwest European. One Italian and two Hungarians were included because the divergences due to their diseased states overshadowed any racial variation.

Photographs and anthropometric measurements constituted the data on build. The methods of measurement, and the calculations of the various indices from them, have been described in the papers cited. Original and derived measurements were plotted on suitable deviation charts, which were then used to analyze the data on build. The pertinent clinical findings are summarized in the tables.

Definition of Build Groups

In an attempt to sort patients into groups according to build, a purely empirical procedure was followed. Various measurements, indices, and dividing limits were tried, until criteria were set up in which the photographs, the anthropometric measurements, and the clinical diagnoses were in reasonable agreement. Eventually four groups were defined: feminine, hypofeminine, hyperfeminine and virile (fig. 1).

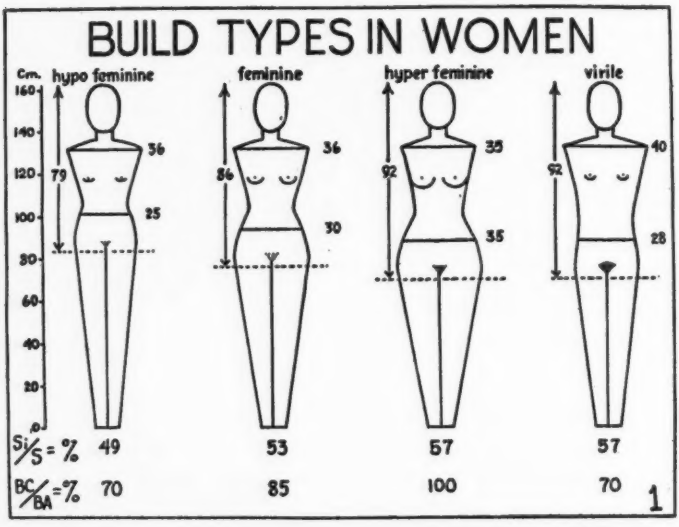
The classification is based on the extent and direction of sexual differentiation as expressed in a selected set of anthropometric characteristics, two descriptive, and two numerical:

a), Breast development.
b), Hair distribution on trunk.
c), Sitting height stature index = sitting height divided by stature = Si/S. This ratio is an accepted means of determining long or short-leggedness. Long-leggedness is the best known anthropometric evidence of failure to mature, because it is evidence of failure of epiphyses to close.
d), Trunk breadth index = bi-cristal pelvic breadth divided by bi-acromial or shoulder breadth = BC/BA. This ratio has occasionally been used as an index for distinguishing typically feminine from typically masculine human forms. In combination with the Si/S it seems very effective for this purpose.

The indices are evaluated in terms of the common statistical measures of means and standard deviations or sigmas, as illustrated in figure 2. In this graph, the two indices, Si/S, and BC/BA are used as coordinates, crossing at their means. The scale is in terms of standard deviations or sigmas (σ). The limits of the groups are indicated by shading.

The 4 groups may be defined with regard to their essential features.

a), Normal feminine: breasts well developed, body hair normally distributed, both indices within one standard deviation or sigma (σ) of the average.



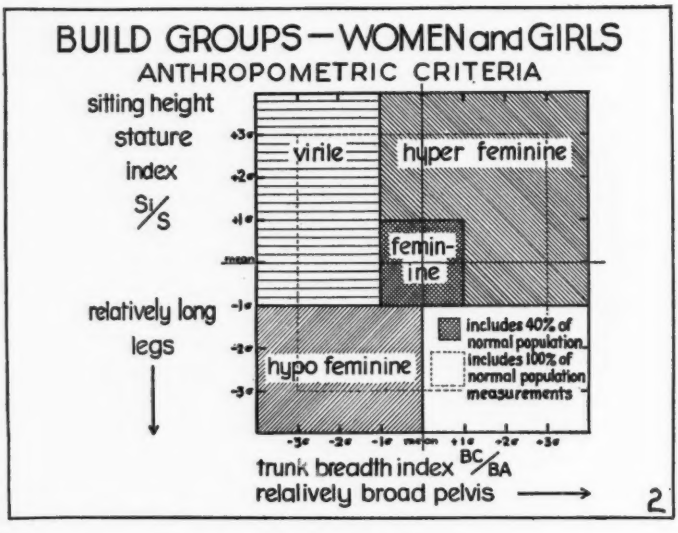


Fig. 1. BUILD TYPES IN WOMEN.
Fig. 2. BUILD GROUPS—WOMEN AND GIRLS.

b), Hypofeminine: breasts small, hair sparse, both indices small, that is, long legs and narrow pelvis. The sitting height stature index is less than one sigma, and the trunk breadth index less than the mean.

c), Hyperfeminine: breasts large, hair dense and sometimes excessive, both indices large, that is, short legs and broad pelvis. One or both of the indices is larger than one sigma.

d), Virile: breasts small, hair excessive, Si/S index within the feminine and hyperfeminine range but the BC/BA index less than one sigma, that is narrow pelvis and broad shoulders.

The actual values for these two indices in working women are as follows

	Mean ± P.E.	Sigma ± P.E.
Si/S	52.99 ± .09	1.30 ± .06
BC/BA	84.86 ± .33	4.88 ± .23

(The ranges limiting each group are shown in table 2.)

TABLE 2. INDEX LIMITS

	Hypo-feminine	Feminine	Hyper-feminine	Virile
Si/S σ scale	-3 to -1	-1 to $+1$	-1 to $+3$	-1 to $+3$
Working women values %	49 to 51.7	51.7 to 54.3	51.7 to 57	51.7 to 57
BC/BA σ scale	-3 to mean	-1 to $+1$	-1 to $+3$	-3 to -1
Working women values %	70 to 85	80 to 90	80 to 100	70 to 80

Definition of build groups is presented in terms of standard deviations rather than absolute values, however, because these groups are conceived primarily as variations of the normal. The classification should thus be applicable to quite different series of patients, as, for instance negroes, if the appropriate means and standard deviations are available. Furthermore, because girls as well as women were considered, it would have been necessary to give at least a score of scales if the absolute values were to be cited for all ages.

As it stands, the classification is applicable to adult women, and girls who have achieved their mature proportions. For younger children, certain factors depending on patterns and rates of development still need to be worked out. It may be noted that when the original 100 normals (working women) were plotted on this graph, 40 per cent fell within the square delimited by the $\pm 1\sigma$ point for both characters, 100 per cent fell within the $\pm 3\sigma$ square. The plot's complete scatter shows absence of relation between the two ratios. This finding is important because, for the particular problem in hand, it was desirable to find two unrelated variables, since two separate factors were sought: maturation (extent of differentiation), and direction of differentiation.

The silhouettes in figure 1 can now be more exactly appreciated by noting that the feminine sketch represents measurements falling at the mean for both Si/S and BC/BA, whereas the other three sketches represent three divergent combinations of the two ratios at outer limits of normal. They are thus rather extreme examples of their respective groups.

Comparison with other Classifications

Although this classification was drawn up with due deference to the typings of previous authors, it does not precisely match any other, probably because its basis is different. Elaborate compilations such as the one by Wertheimer (3), show that many classifications sort out only two great groups, roughly similar to the lineals *vs.* the laterals of Stockard. Among such antitheses are Hippocrates' habitus phthisicus *vs.* apoplecticus, Bauer's asthenics *vs.* arthritics, and the hypo *vs.* hypervegetatives of Pende and the Italian school. Bauer's popularly recognized Rubens type is presumably like our hyperfeminine. Certain of the better known classifications of the French and German schools, however, recognize three or four groupings, comparable to ours. Gläsmer, although she does not discuss virilism, comes nearest to our own observations in both build and clinical considerations. Table 3 shows an attempt to approximate our classification to some of its predecessors.

TABLE 3. DEVELOPMENT FROM PREVIOUS CLASSIFICATIONS

Sigaud 1906 (4)	cerebral		digestive	muscular	respiratory
Jayle 1918 (5)	ovarian, hypo			suprarenal	pituitary thyroid
Kretschmer 1925 (6)	asthenic		pycnic	athletic	
Zondek 1926 (7)	genital		pituitary	suprarenal	thyroid
Gläsmer 1929 (8)	hypoplastic	euplastic	hyperplastic	not considered	
Bayer 1938	hypofeminine	feminine	hyperfeminine	virile	not yet analyzed

Clinical Characteristics

In order to classify patients, their indices were first plotted on the graph shown in figure 2. The 72 of the original 90 cases selected for further analysis are those in whom the photographs showed that hair development and breast size were compatible with their proportions. The size of the breasts was considered especially important, and no patient was classified as hypofeminine or virile if she had large breasts. Where all 4 criteria did not substantiate each other, patients were for the present put in a 'mixed' class and left for a later study.

Although the limits of the build groups were originally fixed with some reference to diagnosis, their real plausibility was now tried by an analysis of clinical findings, which are summarized for each group in tables 4 and 5. Where possible, some statistical statement is made. Where the information does not lend itself to statistical treatment, either because of its nature or because of the small number of observations, an indication is made of the most frequent pathological tendency. The differences in the metabolic rates

TABLE 4. DISTRIBUTION, AGES, AND ANATOMICAL TENDENCIES

	Hypo-feminine	Feminine	Hyper-feminine	Virile	Mixed
Number	9	20	31	12	18
Age means yr.±S.E.	22.0±3.6	31.9±2.4	24.5±1.9	25.0±3.1	26.1±2.5
Weight/S	—	n	+	±	
Fat distribution	n	n	girdles, both	girdles, upper	
Trunk hair	—	n	n or +	masculine dist.	
Breasts	—	n	+	—	
Uterus	—	n	n	— clitoris+	

n=normal
±=unrelated
+=increased, enlarged
−=decreased, small

are not statistically significant. Neither are the differences for the ages of menarche between feminine, hyperfeminine, and virile groups, although an enlarged series may prove the result here suggested—that the hyperfeminine and virile groups mature earlier. A crucial finding is the fact that 5 out of 9 patients in the hypofeminine group, with a mean age of over 25 years, had not yet menstruated. Certain other facts are not recorded: visual field and

TABLE 5. PHYSIOLOGICAL TENDENCIES

	Hypo-feminine	Feminine	Hyper-feminine	Virile
B.M.R. mean %±S.E. (No.)	−6.0±4.2 (8)	−7.8±2.7 (19)	−3.0±2.2 (30)	−3.7±3.5 (12)
Sugar tolerance (No.)	+ (4)	± (6)	n or − (15)	± (5)
Bone age (No.)	− (5)		+ (4)	
Menarche mean age±S.E. (No.)	25.4±6.6 (5)[1]	13.2±0.4 (19)	12.8±0.3 (29)	12.7±0.5 (12)
Type of menstrual disorder	amenorrhea, primary	disturbed rhythm or flow		amenorrhea, secondary

[1] Mean age of the 5 patients in the group who have not yet menstruated.
n=normal ±=unrelated +=increased, accelerated −=decreased, retarded (No.)=number of observations

fundi examinations were made without yielding useful information. Blood studies for estrin and pituitary hormone, together with endometrial biopsies, were made on the cases studied with Dr. Fluhmann, and will be reported

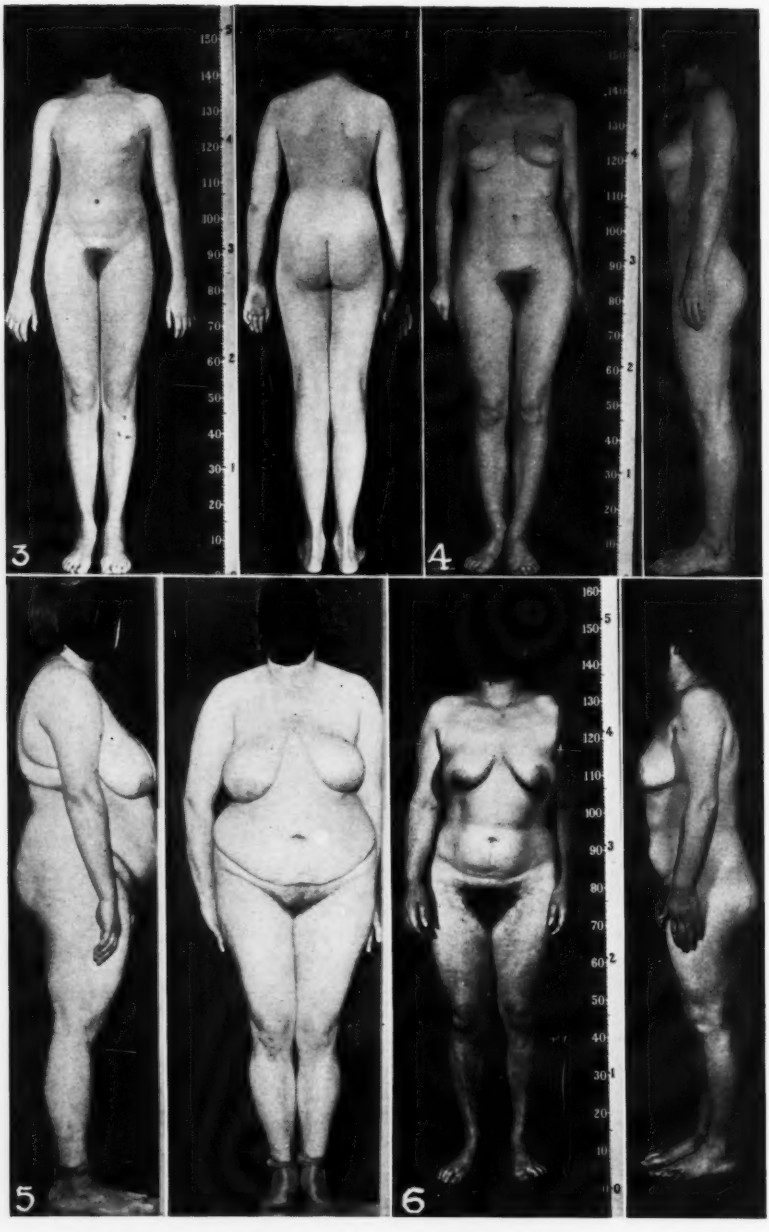


Fig. 3. Hypofeminine. Age 20; amenorrhea, primary.
Fig. 4. Feminine. Age 34; hypermenorrhea, dysmenorrhea.
Fig. 5. Hyperfeminine. Age 24; oligomenorrhea; obesity.
Fig. 6. Virile. Age 20; amenorrhea, secondary; obesity; dermoid cyst or ovary.

separately by him. Measurements on x-rays of the sella turcica will also be reported later.

With particular attention to the problems of menstrual disorder and obesity, the tables permit a clinical characterization of the 4 groups. It must be emphasized that the syndromes are submitted as typical, but that they are not often complete.

a), Feminine: essentially normal; menses disturbed only as to rhythm and flow.
b), Hypofeminine: underweight; amenorrhea, primary; incomplete genital development, delayed bone age, increased sugar tolerance.
c), Hyperfeminine: overweight; menses disturbed also in rhythm and flow; advanced bone age, decreased sugar tolerance. The fat in all 3 groups is deposited especially in both girdle regions: the typically feminine distribution.
d), Virile: overweight and muscular; amenorrhea, secondary; masculinization of the genitals. Fat especially in the upper girdle.

Three of these types lie in a straight line of succession: hypofeminine→feminine→hyperfeminine; Venus of Cranach→Venus of Milo→Helene of Rubens. If the feminine build represents the balanced female biotype, then the hypofeminine woman is one who matures incompletely, and the hyperfeminine woman mirrors an overripening. Contrasted with these two variations, the virile woman is one who matures but in a heterosexual direction.

Illustrative Cases: Many patients presented striking examples of agreement between symptoms and build. For instance, the one case in the series of markedly precocious menarche —a girl who menstruated at 7 years of age, had at 13 years of age unusually short legs and broad pelvis for her age, that is, her indices put her in an extreme hyperfeminine position Similarly the measurements of the 3 adult cases of primary amenorrhea placed them in the hypofeminine group, and the only 3 women in the whole series who underwent a surgical exploration for tumor because of virilism, had measurements which were in the virile area. Figures 3, 4, 5 and 6 demonstrate the 4 build types in women having compatible clinical records. All are nullipara.

SUMMARY

In search of a relation of physique to menstrual disorders and obesity, 4 build groups in women and girls have been sorted out. They have been defined in terms of anthropometric criteria which were intended to reflect extent and direction of sexual maturation. These groups have been called hypofeminine, feminine, hyperfeminine, and virile.

The clinical testimony tends to corroborate the classification both in its lines of demarcation and in its developmental thesis. It has therefore been possible to attempt a characterization of each group not only as to build picture, but also as to typical anatomical and physiological corollaries. *a*), Normal feminine: Build is distinguished by normal body hair and breast development, and average proportions, especially with regard to sitting height-stature and trunk-breadth indices. Clinically these patients are usually normal except that there may be menstrual disturbances of flow and rhythm. *b*), Hypofeminine: Build is distinguished by scant body hair, small breasts, long legs, narrow pelvis. Underweight, primary amenorrhea, incomplete genital development, delayed bone age, and increased sugar tolerance constitute the associated syndrome. *c*), Hyperfeminine: Build is dis-

tinguished by increased body hair, large breasts, short legs, broad pelvis. Overweight, advanced bone age, decreased sugar tolerance constitute the clinical syndrome. Menstrual disturbances consist in irregularities of rhythm and flow. *d*), Virile: Build is distinguished by excessive body hair, small breasts, short legs, broad shoulders, narrow pelvis. Overweight, muscularity, secondary amenorrhea, masculinization of the genitals constitute the extreme clinical syndrome.

REFERENCES

1. Gray, H., and J. G. Ayres: Growth in Private School Children. University of Chicago Press, Chicago, 1931.
2. Bayer, L. M., and H. Gray: *Human Biol.* 6: 472. 1934.
3. Werthheimer, F. I.: *Medicine* 5: 375. 1926.
4. Sigaud, C., and L. Vincent: Les Origines de la Maladie. Maloine, Paris, 1906. (quoted by Jayle)
5. Jayle, F.: L'Anatomic Morphologique de la Femme. Masson et Cie. Paris, 1918.
6. Kretschmer, E.: Physique and Character. Harcourt Brace and Co. New York, 1925.
7. Zondek, H.: Die Krankheiten der Endokrinen Drusen. Julius Springer. Berlin, 1926.
8. Gläsmer, E.: *Ztschr. f. Geburtsh. u. Gynäk.* 96: 572. 1929.

CASE REPORTS

A SYNDROME CHARACTERIZED BY PREMENSTRUAL DERMATOSIS, POLYHYPERMENORRHEA, DYSMENORRHEA, MENSTRUAL HEADACHE AND AN INCREASED URINARY EXCRETION OF SODIUM PREGNANEDIOL GLUCURONIDE

E. C. HAMBLEN

From the Endocrine Division of the Department of Obstetrics and Gynecology, Duke University School of Medicine and Hospital

DURHAM, NORTH CAROLINA

THE FOLLOWING case record is reported in detail because of the apparent association of an increased urinary excretion of sodium pregnanediol glucuronide (metabolic product of progesterone) with the symptomatology of the patient and because of the subjective relief afforded the patient by intensive endocrine therapy which depressed the metabolism of progesterone and decreased the urinary titers of the pregnanediol complex.

CASE REPORT

A white married woman, aged 27 years, para 0-0-0, was seen first in the Endocrine Division of Duke University Hospital on March 4, 1938 because of a cyclic dermatosis occurring during the progestational stage of the menstrual cycle, polyhypermenorrhea, dysmenorrhea and menstrual headache of approximately one year's duration.

No significant familial data existed. There had been no abnormalities of somatic development or of sexual maturation. Menarche occurred at 14 years of age. Menses had been cyclic and of normal duration until the onset of the present illness. The past health had been good. There had been no serious illnesses. She had had an appendectomy with uneventful convalescence 5 years before. The patient had been married for 5 years. There had been no pregnancies.

Present Illness. The patient's menstrual cycle had been considered normal from the time of menarche at 14 years of age until she went in training for nursing at 21 years of age. For the next 4 years she had had marked irregularities, characterized by episodes of amenorrhea lasting at times for 7 months and by occasions of prolonged flowing, which had continued in one instance for 6 weeks and had required vaginal packing. About three years ago, menstruation had become rather well established on a 27 to 30-day cycle with flowing lasting 4 to 5 days.

One year before the patient was seen first by us, in the early Spring of 1937, the interval became shortened to 21 to 24 days and the duration of the flowing increased to 8 to 9 days. The amount of blood loss was considered excessive; the patient reported that a total of from 50 to 60 well saturated napkins were used at each menstruation. Coincident with this alteration of the menstrual cycle, the patient began

to have severe dysmenorrhea and menstrual headache. The dysmenorrhea was characterized by sharp cramping, more marked in the right lower quadrant for the first 24 hours of flowing. Frontal headache began with menstruation and continued for the first two days of bleeding.

On April 21, 1937 the patient was admitted to the Surgical Service of Duke University Hospital because of severe cramping pains in the lower abdomen of 4-days duration. The pain had occurred about one week in advance of menstruation. It had begun in the right lower quadrant of the abdomen and soon became diffusely referred to the entire abdomen. There were radiations to the right costal margin and costovertebral angle and to the region of the right scapula. There had been nausea and vomiting at the onset of the pain. There had been no bloody or tarry stools or hematuria.

Soon after the pain had begun, the patient noticed discrete, flat lesions of purpuric nature which ranged in diameter from 0.5 to 1.5 cm. and which were scattered sparsely over the upper arms and thighs. These had been painful when they first appeared but gradually faded to leave copper-colored discolorations. As the first lesions faded, new ones had appeared. Except for the dermatosis, nothing significant had been found on physical examination save for moderate tenderness in the lower abdomen and about the right costo-vertebral angle.

Special laboratory studies, made at that time, included intravenous pyelography and roentgenologic studies of the gall bladder and gastro-intestinal tract, which gave negative findings. Hematologic data were as follows: hemoglobin 12.5 gm. (80 per cent by the method of Sahli); erythrocytes 3,720,000; leukocytes 3,400; mean corpuscular volume 99 cubic micra; reticulocytes less than 1 per cent; platelets 300,000; bleeding-time 1¼ minutes; clotting-time 4 minutes (Wassermann tube); the differential partition of leukocytes was normal. A Friedman test for pregnancy gave negative results.

It had been the opinion of several consultants at that time that the patient had a non-thrombocytopenic purpura with dermal and abdominal manifestations. The patient was discharged from the hospital after 6 days of observation and study.

The course of the patient after hospitalization was characterized by a continuation of all of these symptoms in association with the progestational stage of the menstrual cycle. The abdominal pain was thought to be a severe dysmenorrhea which began several days before flowing and lasted for about the first 24 hours of menstruation. The polyhypermenorrhea increased. The dermatosis recurred each month about 10 days before an expected period and practically disappeared when bleeding began. There was no menstrual edema associated with these purpuric lesions. They continued to appear in 'crops' for about 6 to 7 days and were painful and itchy for about the first 24 hours. Treatment embracing oral calcium preparations and injections of extracts of corpus luteum proved to be of no value.

Physical Examination. At the first visit to the Endocrine Division on March 4, 1938, physical examination yielded the following data. Temperature was 37° C., pulse 72, respiration 18, height 67.8 in., weight 150.8 lb. and blood pressure 120/80. A general survey showed no somatic or sexual signs of endocrine disease. The patient was of large frame and excellently feminized. The only significant findings on examination (5 days prior to menstruation) were the skin manifestations. On the lower extremities, especially about the knees, were 4 or 5 flat diffuse ecchymotic areas, varying in diameter from 1 to 2 cm. These showed various stages of effusion. Several were recent and tender.

Anthopometric measurements were as follows.

	Patient	Average Minimum	Average Maximum	Optimal (after Engelbach)
Height	67.8	61.9	66.1	—
Weight	150.8	—	—	136
Span	72	62.5	67.1	—
Upper measurement	33.5	30.7	33.5	—
Lower measurement	32.5	30.5	33.3	—
Head	22	—	—	21.9
Chest	37	—	—	31.0
Abdomen	31	—	—	25.7
Hips	42	—	—	—

Laboratory Findings. Hemoglobin was 98 per cent, erythrocytes 4,300,000 and leukocytes 5,000. Blood smears showed nothing morphologically abnormal. Leukocytes were partitioned as follows: polymorphonuclears 61 per cent, small lymphocytes 29 per cent, transitionals 6 per cent and eosinophils 3 per cent. Urinalysis was normal.

Special Studies. Initiation of therapy was postponed until studies of urinary titers of sodium pregnanediol glucuronide could be made during an entire menstrual cycle.

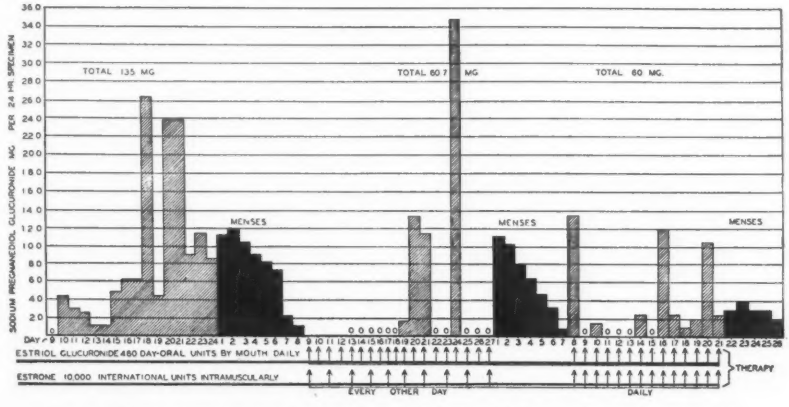


Fig. 1. Urinary excretion of sodium pregnanediol glucuronide in a patient with menstrual dermatosis before and after estrogenic therapy.

The accompanying chart (fig. 1) shows the daily urinary excretion of the pregnanediol complex during 3 cycles. During the last two of these cycles, active estrogenic therapy, as charted, was given and its effect on the urinary titers is shown. The period prior to the first cycle shown in the chart began March 9 and ended March 15 and required 49 well saturated pads. The menstrual cycles shown in the chart corresponded respectively with the next three periods: April 2 to 9, requiring 62 well-saturated napkins; April 28 to May 4, 44 napkins; and May 22 to 26, 15 napkins.

As the chart indicates, there was a beginning excretion of the pregnanediol complex on the second day after period which began March 9th had ceased. This excretion was continuous until the onset of bleeding, April 2nd. A total of 135 mg. of sodium pregnanediol glucuronide was excreted during this time.

A biopsy of the endometrium was obtained on April 3rd, 20 hours after the onset of bleeding. The endometrium secured at this time showed a normal menstrual pattern in which, as yet, no sloughing had occurred.

Treatment and Course. In view of the large amount of sodium pregnanediol glucuronide being excreted in the urine, intensive estrogenic therapy was started and given as shown in the chart. This was designed to depress the function of intercurrent corpora lutea and, thereby, it was hoped to modify the symptomatology by decreasing the metabolism of progesterone, i.e. the urinary titers of the pregnanediol complex. Decreases did occur in these titers. During the first cycle of therapy only 60.7 mg. of sodium pregnanediol glucuronide was excreted and during the second cycle 60 mg. was excreted.

During the first of these cycles, the patient was treated during the interval between bleeding. She was given 10,000 I.U. of estrone (theelin) intramuscularly every other day and 480 day-oral units of estriol glucuronide (emmenin) orally each day. During the second cycle of therapy, the estrone was given daily during the non-bleeding phase, and the estriol glucuronide was continued as before.

Symptomatic responses also occurred. The decrease in the amount of bleeding after the first two series has been described. The menstrual cycle was lengthened. The dysmenorrhea was reduced markedly and headaches did not occur. During the first series of therapy only two small lesions appeared and during the second series, three were observed.

After these two periods of therapy, the patient moved to a distant part of the country, making impossible further studies of urinary titers of the pregnanediol complex. Treatment with oral estriol glucuronide was continued. The patient has reported her progress as follows. Menses occurred from June 17 to 23, requiring 12 napkins (duration of cycle 27 days); from July 11 to 16, requiring 12 napkins (duration of cycle 24 days); and from August 6 to 11, requiring 11 napkins (duration of cycle 26 days). During these three cycles, no headaches occurred, dysmenorrhea was absent and none of the ecchymotic lesions was observed.

A communication in December from the patient reported the recurrence of her various symptoms following discontinuation of oral estriol glucuronide during the preceding month.

DISCUSSION

The excretion of 135 mg. of sodium pregnanediol glucuronide during the menstrual cycle, prior to the beginning of therapy was considered abnormal. Our group (1) has not encountered any values this high in healthy women having cyclic menstruation. The method, employed for these determinations, is essentially the one described by Venning (2) and our normal values have been in rather close agreement with the average total of 45 to 55 mg., reported by Venning and Browne (3).

Studies of the urinary titers alone do not permit an assignment of the cause of these increased values. They may represent an increased formation of progesterone or an increased metabolism and utilization of progesterone formed by ovaries functioning at normal levels. An increased renal permeability to the pregnanediol complex might result in similar findings.

It is unfortunate that gonadotropic and estrogenic titers of the urine were not studied. These, perhaps, would have supplied additional significant data.

At the present time it is not possible to make a definite clinical estimation of the complete metabolism of progesterone, i.e., determinations of the total progesterone formed by the ovaries, of the amount metabolized and excreted

as sodium pregnanediol glucuronide, and of the amount destroyed or excreted unchanged or in some other conjugated form. Practical methods likewise are not available for studying the titers of progesterone or its metabolic products in the blood.

The author is inclined to believe that an increased formation of progesterone was responsible for the high titers of the pregnanediol complex. The effect of the subsequent estrogenic therapy upon these seems to justify this view to some extent, since the primary effect of the treatment probably would be a depression or inhibition of the function of the corpora lutea.

The association of the symptomatology with the progestational phase of the cycle seemed correlated with the hormonal findings and the relief of these symptoms by therapy which altered the abnormal endocrine condition would suggest that the etiologic factor was an aberration in the metabolism of progesterone.

An explanation of the functional pathology responsible for the individual symptoms is difficult at present. The author suggests, however, that the dermal effusions producing the ecchymotic lesions might well be due to alterations in capillary permeability brought about by the increased metabolism of progesterone. A cyclic effect of this nature occurs in the spiral arterioles of the endometrium and seems related etiologically to the action of ovarian sterols.

The investigation of hormonal titers in other patients, who exhibit dermatoses during the progestational phase of the menstrual cycle, may lead to a clearer concept of the interrelationship to ovarian functions and in particular to that of the corpus luteum. The patient described apparently does not fall into the same classification of those who have menstrual edema associated with cyclic dermatoses, since it seems reasonable to relate the edema to a retention of the sex sterols rather than to an increased excretion.

SUMMARY

A patient was described whose symptoms included cyclic ecchymotic lesions during the latter part of the progestational phase of the cycle, polyhypermenorrhea, dysmenorrhea and menstrual headache. She was found to excrete an abnormally large amount of sodium pregnanediol glucuronide in the urine. Intensive estrogenic therapy reduced these abnormal urinary titers and resulted in the disappearance of all the symptoms. An increased formation and metabolism of progesterone was thought to be related to the symptomatology of the patient.

The author acknowledges the technical assistance of Mrs. Catharine Ashley who made the studies of the urinary titers of sodium pregnanediol glucuronide. Some of the expenses incurred in these studies were defrayed by means of a grant from the Research Council of Duke University. The estrone (theelin) used was supplied by Parke, Davis & Co., Detroit, Mich. The estriol glucuronide (emmenin) was furnished by Ayerst, McKenna and Harrison, Montreal, Canada.

REFERENCES

1. Hamblen, E. C., C. Ashley and M. Baptist: *Endocrinology* 24: 1. 1939.
2. Venning, E. H.: *J. Biol. Chem.* 119: 473. 1937.
3. Venning, E. H., and J. S. L. Browne: *Endocrinology* 21: 711. 1937.

GYNECOMASTIA[1]

I. P. BRONSTEIN

From the Department of Pediatrics, University of Illinois College of Medicine and the Research and Educational Hospital

CHICAGO, ILLINOIS

GYNECOMASTIA is the occurrence of feminine breast tissue in the male. Our patient, a colored boy, 17 years of age (fig. 1), presented the less frequent bilateral mammary development resembling that of the female in gross physical appearance. The breasts were neither painful nor inflamed and secreted no milk. The gynecomastic breast may secrete milk if the proper stimulus is provided and histological study reveals little if any difference from the young virginal or nulliparous female. Castrated young male rats and guinea pigs in whom ovaries from consanguinous animals have been implanted develop physical and psychical characteristics of the female, including lactating mammae for nursing the young. The male genitalia in these animals become infantile.

The mode of development of the mammary gland up to puberty is similar in the male and female. After this period marked quantitative differentiation occurs in the latter, presumably due to ovarian secretion, since breast development does not take place in ovariectomized animals. Virginal hypertrophy of the breast is seen not uncommonly in boys between the ages of 14 and 18 when a tumor-like enlargement occurs behind the nipple undergoing regression in a few months. Extremes of mammary swelling are compatible with normal genital development.

Gynecomastia in most instances has its inception at or about the time of puberty. Clinically, we assume this anomaly to be associated with endocrine dysfunction since this phenomenon is frequently accompanied by defective genitalia, tendency to femininity and a lack of sexual desire. Secondary sexual characteristics such as hair growth may be normal. Our patient had pubic and axillary hair but none on the face or chest.

The breast enlargement had been present for about 5 years. In addition, there was mental retardation first noted at the age of 10 when a test revealed a mental capacity of 7 years. The penis and scrotum were normal. Both testes were definitely undersize and firm containing no tumor mass or irregularity. The left measured 2.5 × 1 cm. and the right 3 × 2 cm. There was no external evidence of an hermaphroditic condition. The epididymis appeared normal but was small in outline. There were no inguinal herniae or masses. The prostate was represented by only a slight thickening of tissue between the symphysis. The seminal vesicles were not palpable and massage of these yielded no fluid. Spermatozoa were not found in the urine voided following this procedure. The emotional reactions, mental trend and general contour were

[1] Presented at the American Academy of Pediatrics (Region III), Oct. 15, 1937.

effeminate. The hips were wide and the fingers long and tapering. The boy did not masturbate and had no sex curiosity, seminal emissions or erections.

The height was 177.5 cm., the weight 66.59 kg., and the span 188 cm. The lower measurement from the pubic eminence to the ground level was 94 cm., the upper to the cranial vertex 83.5 cm. His head appeared small, the occipital-frontal measuring 54.5 cm., the chest below the breasts was 78 cm., and the circumference of the abdomen at the umbilicus 70 cm. The B.M.R. was normal, there were no symptoms to suggest an excessively functioning thyroid gland. Occasionally an hyperthyroidism may produce gynecomastia.

The clinical and postmortem observations in gynecomastia are variable and confusing. In the embryo the testicle and ovary coexist with a tendency to bisexual development. If, for some reason, the testes fail to develop normally, whether due to an injury or anomaly, the normal inhibitory influence of testicular secretion upon estrin in the male is withdrawn. This may be seen in damage by x-ray, syphilis, tuberculosis or mumps, any of which may cause partial or complete destruction of the testes. In our patient the Kahn test finding was negative, there was no history of parotitis nor any other predisposing factor which might have influenced the condition. The atrophy following orchitis resulting in gynecomastia may support the theory of testicular secretion inhibiting male breast growth. Attention has been called to the high content of estrogenic substance in breast tumors and the implication advanced that in gynecomastia the abnormality may be ascribed to estrogenic activity. There may be present, therefore, a high secretory level of estrogenic hormone in gynecomastics producing secondary sexual characteristics of the female. In hermaphrodites where ovarian tissue is found excessive production of estrogens occurs.

FIG. 1.

In an earlier study, estrogenic activity was present in one patient with gynecomastia but not in another. In a more recent work no unusual amount of estrogenic substance was obtained in gynecomastia, thereby lending no support to the theory of hyperestrinism. In this latter work the amount of androgen found was variable. Estrin assay of the urine of our patient was negative; male sex hormone determinations were not made.

Suprarenal cortical tumors may cause gynecomastia by depressing the secretion of the testes. In this connection a recent report on the production of gynecomastia, in an adult with Addison's disease, by the use of adrenal

cortex extract is interesting. There have been few proven cases of adrenal cortical tumor in the male causing feminization with gynecomastia and lactation. Examination of the abdomen in this case was negative as was also the flat x-ray picture. There were no urinary symptoms and the result of the urine examination was not significant. Genito-urinary visualization was not done and air injection to visualize the adrenal was not attempted.

Pituitary and pineal tumors may cause gynecomastia. Results of examination of the fundus and visual field study were negative. X-ray examination of the head showed a rather flat sella turcica, apparently bridged over. No definite pathologic significance was attached to this finding. A small density was present in the pineal region representing calcification in this structure. The films of the knee and wrist exhibited no pathology and ossification was approximately normal for age. The sugar tolerance was normal. There were no signs or symptoms, whatsoever, of intracranial involvement.

A better understanding of breast conditions may be possible when a suitable test for prolactin, the lactogenic hormone derived from the anterior pituitary, is devised. This factor apparently acts on fully prepared mammary tissue, principally to initiate milk secretion. Some investigators report an unidentified AP factor which favors mammary growth apart from the prolactin. In this connection it may be mentioned that chorion-epitheliomas of the testes apparently inhibit the prolactin secreting cells of the pituitary since only a small proportion of these cases exhibit gynecomastia. In this condition tremendous amounts of prolan, much in excess of that which is necessary to give a positive pregancy test, are found.

Of particular interest was the finding of a positive Friedman test (Reaction III) which remained positive after the patient had had therapy.[2] The exact interpretation of the excess of urinary prolan is conjectural but, nevertheless, important from the point of view of pituitary physiology. Does the excessive prolan excretion found here signify primary pituitary dysfunction leading to abnormal mammary hyperplasia, in this instance, without lactation? Is the deficient testicular development responsible for this excess? In eunuchoidism the excretion of testicular hormone may be only one-tenth the normal, and the APL substance is found in great quantities in the urine. The evaluation of gynecomastia in terms of hormones is as yet very difficult since the data on the excretion of these substances in this condition is scant.

Replacement therapy was employed, the indication being the apparent testicular insufficiency. Sixty injections of male sex hormone were given.[3] Inasmuch as no positive assertions are warranted as to the results, the following suggestive changes occurred: erections; appearance of hair on the chest, face, and legs; very noticeable interest in the opposite sex; enlarge-

[2] The Friedman test (Reactions II and III) is a qualitative method for gonadotropic hormone assay, indicating excessive quantities of urinary prolan but does not differentiate definitely between prolan A and B. Normally in children little if any gonadotropic hormone is found, in young adults from 4 to 19 mouse units have been assayed. In castrates there is a marked increase of this substance.

[3] Hydrosoluble fraction from spermatic gland plus liposoluble principle from interstitial gland (144 gm. of fresh gland) and testosterone propionate (210 mg., approximately 10,500 I.U.). Material supplied by Ciba Company.

ment of both testes; and questionable shrinkage of the right breast. The above changes remained for several months following the last treatment, after which time the patient failed to report.

REFERENCES

1. Hammett, F. S.: *Endocrinology* 4: 205. 1920.
2. Glandular Physiology and Therapy, American Medical Association, 1935.
3. Geschickter, C. F., D. Lewis and C. G. Hartman: *Am. J. Cancer* 21: 828. 1934.
4. Lewis, D., and Geschickter, C. F.: *Ann. Surg.* 100: 779. 1934.
5a. Gallagher, T. F., D. H. Peterson, R. I. Dorfman, A. T. Kenyon and F. C. Koch: *J. Clin. Investigation* 16: 695. 1937.
5b. Kenyon, A. T., D. H. Peterson and T. F. Gallagher: *J. Clin. Investigation* 16: 705. 1937.
6. Katzman, P. A., and E. A. Doisy: *J. Biol. Chem.* 106: 125. 1934.
7. Edwards, R. A. M. B. Shimkin and J. S. Shaver: *J.A.M.A.* 111: 412. 1938.
8. Wolf, W.: Endocrinology in Modern Practice, Saunders, Philadelphia, 1936.

CLINICAL AND LABORATORY NOTES

THE CONCENTRATION OF A VOLATILE REDUCING SUBSTANCE, PROBABLY ALCOHOL, IN BLOOD AND URINE OF DIABETIC PERSONS

HARRY BLOTNER*

IN STUDYNG the alcohol in the blood and urine of normal individuals, Gibson and Blotner[1] found that the normal ethyl alcohol concentration in the blood varied from 0 to 6.5 mg. per cent and in the urine from 0 to 2.4 mg. per cent. Since the method used for determining alcohol is not specific because a volatile reducing substance such as acetone gives the test, it appeared to be of clinical interest to compare with the normal the results that may be obtained in the blood and urine in diabetic patients who are subject to ketosis, and to observe any relation between the alcohol or volatile reducing substance, the sugar concentration in the blood and urine and the use of insulin. This paper reports the results obtained in 46 patients with diabetes mellitus with no acetonuria, 5 patients with diabetes insipidus, 1 patient with alkalosis, and 3 diabetics and 3 nephritics with acidosis.

Experimental Procedure. The alcohol and sugar concentrations were determined in specimens of blood and urine taken simultaneously from a group of diabetics (fasting or non-fasting) who had had no alcohol for several days at least. The method used for determination of the alcohol is that described by Gibson and Blotner, which employs the Evelyn photoelectric colorimeter. In determining the normal alcohol 10 cc. of the Folin-Wu blood filtrate and 10 cc. of urine were used for distillation. The skin was sterilized for venepuncture with a 1–1000 bichloride solution. Twenty-four of the patients were being treated with insulin. The blood sugar varied from 90 to 400 mg. per cent and the urinary sugar from 0 to 4 per cent whether or not insulin was administered.

Results. The results[2] obtained in 46 diabetics without acidosis were interesting because they were much the same as those observed in normal individuals. Of these diabetics, 29 had no alcohol or volatile reducing substance in the blood while 17 had from 1 to 6.5 mg. per cent. Simultaneous urine samples, obtained in 30 of these cases, showed no alcohol or volatile reducing substance in 6 cases and from 0.1 to 2.0 mg. per cent in 24 cases, the usual amount being less than 1 mg. per cent. The urine alcohol concentration was practically the same whether or not alcohol was present in the blood. The use of insulin and the amount of sugar in the blood and urine had no relation to the concentration of alcohol or volatile reducing substance in these fluids.

It is possible that there may have been minimal amounts of alcohol in the specimens which showed none because it was found, with known concentrations of alcoholic solutions, that 1 or 2 mg. per cent was not recovered at all times, probably because it was lost in the process of distillation.

* From the Medical Clinic of the Peter Bent Brigham Hospital, Boston, Mass.

[1] GIBSON, J. G., 2ND. AND H. BLOTNER: *J. Biol. Chem.* 126: 551. 1938.

[1] BLOTNER, H.: *New England J. Med.* In press.

[2] Miss Y. L. Dondos gave technical assistance.

Of the 5 patients with diabetes insipidus who were studied, 3 showed no alcohol in the blood and urine, whereas two had 4 to 6.5 mg. per cent in the blood and less than 1 mg. per cent in the urine. Since this method is not specific for alcohol, the test was made in 3 other patients with diabetes mellitus who had varying amounts of acetone or diacetic acid in the urine. These specimens of urine gave the test and showed a concentration of 149, 15 and 21 mg. per 100 cc. of urine. The patient with the last urine had in the blood a volatile reducing substance concentration of 55 mg. per 100 cc. No doubt these high values were due to the ketone bodies of diabetic acidosis.

Finally, in order to ascertain whether a different form of acidosis would give this test, 3 patients with nephritic acidosis were selected. Their blood urea nitrogen concentration ranged from 124 to 150 mg. per cent. The blood alcohol in these patients was normal and varied from 0 to 6.5 mg. per 100 cc. Incidentally, a patient with alkalosis and a blood CO_2 of 104 volumes per cent showed no alcohol in the blood. In testing for alcohol in diabetes mellitus, it appears important to remember that acidosis will give this test and that it may be confused with alcoholism if the latter were suspected.

Summary. This paper presents observations on the concentration of alcohol or volatile reducing substance in the blood and urine of 46 patients with diabetes mellitus without acidosis, 5 patients with diabetes insipidus, 1 patient with alkalosis, and 3 diabetics and 3 nephritics with acidosis. The concentration of alcohol or volatile reducing substance in the blood of these people was the same as in the normal and varied from 0 to 6.5 mg. per cent and in the urine from 0 to 2 mg. per cent except in the diabetics with acidosis. In these the readings were considerably elevated and apparently due to ketone bodies. There was no relation between the amount of sugar in the blood and urine and the concentration of alcohol or volatile reducing substance in these fluids.

189 Bay State Rd.
Boston, Mass.

ASSOCIATION NOTICE

ANNOUNCEMENT OF THE ANNUAL MEETING

THE TWENTY-THIRD annual meeting of the Association for the Study of Internal Secretions will be held in St. Louis, Missouri, on May 12 and 13, 1939. The Hotel Jefferson at Twelfth and Locust Streets will be the headquarters for registration and for the scientific and business sessions.

The Chairman of the Local Committee is Dr. Daniel L. Sexton, University Club Building, St. Louis.

Hotel accommodations may be reserved directly with the hotel management for the period covered by our meeting.

PRESENTATION OF PAPERS

Members of the Association and other investigators who wish to present papers will please note the following regulations regarding the program for the coming meetings, especially since they differ in some respects from those in former years.

1. The title of the paper, and a comprehensive abstract *in triplicate*, must reach the President, David P. Barr, M.D., 600 South Kingshighway, St. Louis, Missouri, not later than March 1, 1939.
2. Non-members who present papers will appear on the program "by invitation." Non-members are therefore requested to have their titles and abstracts introduced by a member.
3. The abstracts of papers will be considered by the program committee and the final program will be announced about April 1, 1939.
4. Papers will be limited to ten minutes for presentation and five minutes for discussion.
5. All papers presented before the Association or read by title are to be submitted, in completed manuscript form, to ENDOCRINOLOGY, either before or immediately after the meeting. It is to be understood, however, that acceptance of a paper for the program does not obligate the Journal to its acceptance for publication.

You are invited to bring this announcement to the attention of your colleagues and associates who have endocrine researches in progress.

NOMINATIONS OF OFFICERS

The revised By-Laws adopted at the last annual meeting of the Association provide that nominations for all elective offices shall be made by a Nominating Committee and forwarded to the Secretary at least sixty days

before the annual meeting. The Nominating Committee for the current year, appointed by the President and accepted by the Council, is as follows:

Dr. F. C. Koch, *Chairman*. 947 East 58th St., Chicago, Illinois

Dr. F. M. Pottenger, 1930 Wilshire Blvd., Los Angeles, California

Dr. Oscar Riddle, Carnegie Institution, Cold Spring Harbor, New York

The By-Laws also provide (Article V, Section 2) that "Any member of the Association may submit nominations to the Nominating Committee for its consideration." Any nominations which members desire to make should be sent to Dr. F. C. Koch by February 15, 1939. The terms of the following officers expire at the time of the annual meeting in St. Louis in 1939.

President-Elect	P. E. Smith (advanced to Presidency)
Vice-President	E. P. McCullagh
Secretary-Treasurer	E. Kost Shelton

Council Members	Publication Board
3 (years)	(3 years)
F. A. Hartman	David P. Barr
E. L. Sevringhaus	F. M. Pottenger
Fuller Albright	

E. Kost Shelton, M.D.
Secretary-Treasurer.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright	Murray B. Gordon	A. E. Meyer
Wayne J. Atwell	Arthur Grollman	Warren O. Nelson
Geoffrey Bourne	Frank A. Hartman	J. P. Pratt
Israel Bram	E. C. Hamblen	A. T. Rasmussen
John C. Burch	F. C. Koch	Elmer L. Sevringhaus
H. J. Deuel, Jr.	Edward Larson	L. B. Shpiner
John C. Donaldson	J. T. Lewis	D. L. Thomson
Maurice H. Friedman	Joseph M. Looney	R. L. Zwemer

BOOK REVIEWS

ENDOCRINE THERAPY IN GENERAL PRACTICE. Sevringhaus, Elmer L., pp. 192, The Year Book Publishers, Inc., Chicago, Illinois, 1938.

This book is offered to the medical profession in the hope that it may be useful in helping "to keep up with general progress in endocrinology, to administer new therapy and make more exact diagnoses in some cases and to reserve judgment and withhold therapy when clinical conservatism still requires it." No attempt is made to present the subject of endocrinology in an adequate, systematic fashion but to afford succinct and accurate summaries of those parts of the subject that can be brought practically to bear on clinical problems.

After an orienting introductory section the various glands are taken up seriatim. The clinical problems centering in each are discussed with special regard to differential diagnosis. Then the therapeutic resources that are available for the treatment of the given disorder are discussed. It is to be commended that squeamishness has been laid aside and various commercial endocrine products discussed on their merits, so far as these are known. The use of vitamin preparations as supplementary therapy, of sedatives and of planned psychotherapy when rationally indicated is advocated. Upon appropriate occasions suggestions for needed therapeutic investigations are made. General case management, prognosis and prevention are also discussed. Two particularly useful chapters are those on diabetes and on obesity. Altogether, the book is practical in the best sense of that much abused word. The physician who is well versed in endocrinology will welcome the opportunity mentally to compare notes with a fellow-expert. For the physician who wants a conservative, constructive and reliable discussion free from unnecessary technicalities, the work will prove both interesting and valuable.

SEXUAL MATURATION AND THE SKELETAL GROWTH OF GIRLS AGE SIX TO NINETEEN. Shuttleworth, F. K. Monographs of the Society for Research in Child Development, Volume 3, No. 5, 1938, published by Society for Research in Child Development, National Research Council, Washington, D. C.

In a set of 8 tables and 42 graphs the author presents the developmental pictures of three groups of girls: those of early, those of late and those of medial maturity. The items measured were: 12 skeletal dimensions; standing and sitting height; leg length; iliac diameter; chest depth and breadth; and weight. Each of the 19 dimensions was found to have its own characteristic increment pattern. Accelerating and decelerating phases occurred in the various features in such combinations as to establish composite patterns characteristic of each of the three groups of girls. An endocrine interpretation is offered. The material presented is obviously of special use to endocrinologists who are studying problems of growth and maturity both as affording anthropometric norms and as permitting differentiation of developmental types.

ADVANCE ABSTRACTS

BERGMAN, A. J., AND C. W. TURNER. A comparison of the guinea pig and chick thyroid in the assay of the thyrotropic hormone. *To appear in this Journal.*

A statistical study is reported concerning the relative value of the day-old male and female white Leghorn chick and the immature male guinea pig in the assay of the thyrotropic hormone of the anterior pituitary. Groups of 20 male and female chicks and 10 male guinea pigs were injected with increasing amounts of an initial extract of the AP. In the chicks the male was found to be more sensitive than the female. For assay purposes 10 male guinea pigs were found to be more reliable than 20 male chicks. The male chick unit of thyrotropic hormone was found to be about ¼ the amount of the guinea pig unit. A chick unit and a guinea pig unit of thyrotropic hormone are defined.

BLUMENFELD, C. M. The effects of ovariectomy on the adrenal glands of the albino rat. *To appear in this Journal.*

The effects of ovariectomy on the adrenal glands of the albino rat were studied in the following groups: 22 control and 21 test animals, operated on at 4 weeks and killed at 3 months of age; 25 controls and 25 tests, operated on at 4 weeks and killed at 6 months; and 4 controls and 3 tests, operated on at 3 and killed at 6 months. In the tests with young groups, the adrenals were significantly smaller due to a decrease in size of the cortex for which diminution of the zona fasciculata was largely responsible. Mitoses were as numerous in the tests as in the controls; fasciculata cell volume was decreased; cell destruction in the reticularis was increased. Increase in size and number of partially and completely pigmented cells in the reticularis was noted. Lipin content was reduced about twice as much as the whole gland was. In the older groups of test rats the changes were essentially similar qualitatively, but greater in degree. Age seemed more important than length of the spayed state in producing the changes. An interpretation offered is this: the cortex, and therefore the whole gland, becomes smaller because its cells are being used up faster than usual due to an increased functional burden placed upon the adrenals by removal of the ovaries.

BRENEMAN, W. R. Augmentation of pituitary extract by chlorophyll, plant growth hormones and hemin. *To appear in this Journal.*

White Leghorn cockerels and 23-day-old female rats were injected with pituitary gonadotropic hormone after admixture in vitro with alcohol or water-soluble chlorophyll, plant growth 'hormones' (indole-butyric acid or l-naphalene acetic acid), or hemin. The action of the pituitary extract was augmented in both the cockerel and rat series. The maximum response was observed in those animals injected with the chlorophyll-pituitary mixtures. The plant growth hormones produced smaller but quite definite increases in ovarian weights of the rat. Hemin added to the pituitary extract had no augmenting effect, but after the addition of a small amount of pyridine to the mixture the action of the hormone was augmented. Cockerels were given a single injection when only 1 day old and were without food or water for 72 hours at the end of which time they were killed. The response of the testes to the various admixtures of hormones and augmenting substances was similar to that observed for the female rat. Testes of chicks injected for 5 days, however, either from the fifth to ninth or from the tenth to fourteenth days, did not evidence the augmentation response. A total of 137 rats and 277 chicks were used in these experiments.

CANTAROW, A., H. L. STEWART AND D. R. MORGAN. Chronic idiopathic parathyroid tetany. *To appear in this Journal.*

A case is reported of severe, chronic idiopathic hypoparathyroidism which did not respond to vitamin D therapy and was effectively controlled by large doses of parathyroid hormone and a high calcium, relatively low phosphorus intake over a period of 5½ years. This regime became ineffective on two occasions, when the only change consisted in an increased intake of phosphorus (and calcium) and vitamin D, and again became effective when the latter were omitted. The significance of this phenomenon is discussed. Death was due apparently to the development of a peculiar hemorrhagic state, with extensive hemorrhages throughout the body. The exact nature and pathogenesis of this state could not be determined; it may have been dependent perhaps upon a condition of prothrombin deficiency. No parathyroid tissue could be found at autopsy. Aside from the hemorrhagic manifestations, the only other significant findings were a mild chronic hepatitis, mild nephrosis, bronchopneumonia and trophic changes in the hair, nails, and teeth.

DYNES, J. B. Clinical pathological report of a patient receiving large dosage of estrogenic hormone. *To appear in this Journal.*

A clinical pathological report of a woman with the syndrome of involutional melancholia who was given large doses of estrogenic hormone and who died six weeks later of other diseases, is given. The

report on the pituitary is of interest if for no other reason than the relatively negative findings after large doses of estrogenic hormone.

FOSTER, M. A., R. C. FOSTER AND R. K. MEYER. Hibernation and the endocrines. *To appear in this Journal.*

The thirteen-lined ground squirrel was found to hibernate more consistently under experimental conditions when dehydrated to a moderate degree. During the breeding season, hibernation could not be induced, but shortly afterward there was maximal tendency to hibernate which persisted until the onset of genital enlargement. From the time vaginal swelling appeared in the female and the testes became scrotal in the male the tendency to hibernate steadily diminished until sexual activity terminated this phenomenon. During this homiothermic or breeding season, extirpation of the pituitary converted these animals into poikilotherms, while removal of the thyroid, adrenal or gonads was without significant effect. Substitution therapy during the non-breeding (poikilothermic) season with pituitary preparations resulted in transient homiothermism. Similar results were obtained with thyroid preparations, but gonadotropic pituitary extracts, corticoadrenal extracts and sex hormones were without effect. These findings suggest inactivity of the pituitary as the primary factor responsible for the phenomenon of hibernation.

GRAY, L. A., AND C. B. BRACK. The effect of estrogenic hormone on experimental tuberculosis. *To appear in this Journal.*

For many years it has been observed that various infections suffer an exacerbation in the premenstrual phase. This has been particularly noticed in tuberculosis. Furthermore the mortality of tuberculosis is greater in women than in men from the age of fifteen to forty-five. It seemed of importance to study the effect of the estrogenic hormone on immunity. Guinea pigs were used to determine the effect of this hormone on native and acquired immunity to experimental tuberculosis. No demonstrable difference was found between the treated animals and the controls. This experiment suggests that the estrogenic hormone has no demonstrable effect on native or acquired immunity to tuberculosis.

GREENE, R. R., M. W. BURRILL AND A. C. IVY. Progesterone is androgenic. *To appear in this Journal.*

Large amounts of progesterone and of progestin given to castrate male rats maintain or stimulate the prostates and, to a lesser extent, the seminal vesicles. These sexual accessories increase in weight with such treatment and display cytological evidence of secretory function. An increase in size of the clitoris can also be obtained by administration of progesterone. From these facts, it is concluded that progesterone is androgenic.

HELLER, C. G., AND E. K. JOHNSON. Gonadotropic hormone, clinical application of extraction methods for assay purposes. *To appear in this Journal.*

Several concentration methods of urinary gonadotropic hormone for assay purposes were compared with untreated urine to test their completeness of recovery. Several consecutive tests were made from the same pooled specimen to test the regularity of results and the methods were varied until the simplest satisfactory procedure resulted. The alcohol-ether concentration method essentially fulfills all requirements of an adequate extraction method for clinical use.

HOUCHIN, O. B., AND C. W. TURNER. The relation of the pituitary to blood lipids. *To appear in this Journal.*

With a single injection of an initial extract of the anterior pituitary into the rabbit, the blood fat was observed to be depressed to the extent of 34 to 36% of the normal blood fat in from 6 to 8 hours after the injection. Similar results were observed in another group of rabbits from which food had been withheld for 24 hours previous to the beginning of the experiment. The fat content of normal controls was observed to remain rather uniform during a corresponding period. Fractions of the initial extract rich in lactogenic hormone and in the carbohydrate metabolism, thyrotropic and gonadotropic hormones injected at the same and at double this level caused no depression of the plasma lipids. Neither adrenal cortical hormone nor thyroxine depressed the plasma fat in the 6 to 10-hour period although thytroxine did begin to depress the fat after 24 hours.

MORATO-MANARÓ, J., AND A. ALBRIEUX. The effect of sex hormones on the combs of castrated and normal cocks. *To appear in this Journal.*

Previous experiments are confirmed concerning the neutral effects (inhibitory effects) of the combination of male hormone plus folliculin on the White Leghorn capon's comb. Folliculin has an influence on the uncastrated hen's comb that is similar, grossly, to the effects on the uncastrated cock's comb. Folliculin has a direct effect on the cock's comb, just as previously reported experiments show. This opens a new field for experimental work in connection with the clinical use of the hormone. The technic used involves dividing the comb.

NATHANSON, I. T., L. E. TOWNE AND J. C. AUB. The Daily Excretion of Urinary Androgen in Normal Children. *To appear in this Journal.*

Utilizing a colorimetric method, the excretion of urinary androgens was determined repeatedly in 4 normal boys and 6 normal girls of varying ages. These studies showed that there was a definite variation in the androgen excretion from day to day, but this was not of an adequate magnitude to be considered physiologically significant. There was no evidence of a cyclic excretion of the androgens in either sex. The excretion varied directly with increasing chronological age and with the physical maturity of the individual. It is suggested that an assay for androgens in a single 24-hour specimen of urine should yield significant information in studies concerning endocrine abnormalities of childhood.

PIZZOLATO, P., AND H. H. BEARD. Creatine-creatinine metabolism and the hormones. Anterior pituitary hormones, theelin and testosterone propionate. *To appear in this Journal.*

Various hormone preparations were injected into normal and castrated rats and the effect of these injections upon creatine-creatinine metabolism was studied. The following results were obtained. *a*), Amniotin (theelin) 200 to 1400 U; perandren (testosterone propionate) 125 to 750 U; and AP extract 5 to 30 U containing the growth, thyrotrophic and sex hormones, caused increases in muscle creatine and in creatine excretion accompanied by decreases in creatinine excretion. Antuitrin-S and T and follutein produced similar but much less striking results. *b*), Castration in both sexes resulted in creatinuria, which was more intense in the female and which was accompanied by a decrease in creatinine excretion. *c*), Injection of all of the hormones listed, with the exception of antuitrin-S and T and of follutein, caused a more significant increase in the total amount of extra creatine excreted in castrated animals than in the normal animals. Antuitrin-G is responsible for the creatinuria during early growth in man. *d*), Removal of the testes did not influence the concentration of muscle creatine but removal of the ovaries and uterus caused significant increases in this constituent. *e*), Hystero-oöphorectomy in 3 patients was followed for 10 days after the operation by a large creatinuria accompanied by a decrease in creatinine excretion. *f*), This inverse relationship between creatinine and creatinine excretion offers irrefutable proof that creatinine is transformed into creatine in the body, and that it stimulates the excretion of both creatine and creatinine in the urine. The transformation of creatine into creatinine does not occur in the rat or in man. g), A possible synergistic action of the hormones in the body on creatine-creatinine metabolism is suggested.

TEEL, H. M., AND D. E. REID. An antidiuretic principle in the urine of pre-eclamptic and eclamptic patients. *To appear in this Journal.*

Concentrates prepared from the urines of pre-eclamptic and eclamptic patients during the phase of acute water retention markedly inhibit water diuresis in rats. This is true of the urines from patients on voluntary water intake as well as from urine specimens collected 3 hours after giving as much as 1400 cc. of water by mouth. In severe cases, antidiuretic effects equivalent to those obtained by injecting 50 or more milliunits of pituitrin are obtained from 8 to 12 cc. of urine. This antidiuretic effect disappears from the urine shortly after delivery. Similarly prepared concentrates from urine obtained during pregnancy and at the time of delivery in normal patients usually show no antidiuretic effect, although occasionally concentrated specimens have given equivocal antidiuresis, comparable to that obtained by 5 to 10 milliunits of pituitrin. However, if patients during normal pregnancy are dehydrated by withholding water for 18 to 24 hours, preparations from the concentrated urine obtained exhibit marked antidiuretic effects, comparable to those shown by severe pre-eclamptics and eclamptics who are on voluntary or forced fluid intake. The antidiuretic factor in potent urine concentrates from patients with pre-eclampsia and eclampsia is insoluble in ether and is destroyed by hydrolysis with NaOH. It is probably not an estrogen and resembles pituitrin.

TURNER, H. H. The clinical use of synthetic male sex hormone. *To appear in this Journal.*

Fifty-four patients with various types of genital anomalies and dysfunctions, which included adult hypogonadism, male climateric, adiposogenitalism, gynecomastia, and benign prostatic hypertrophy, were treated with the synthetic male sex hormone, testosterone propionate. In doses of 10 to 75 mg. weekly, it was found effective in every hypogonad case, as evidenced by promoting hair and penile growth, producing erections and emissions, developing the libido and potentia, and by generalized changes in secondary sex characteristics. Gynecomastia with normal secondary sex development is apparently not influenced by injections of the hormone. The androgen brought about considerable relief of the subjective symptoms of male climateric, and in cases of benign prostatic hypertrophy, as shown by the clinical improvement in the usual symptoms and signs of prostatic obstruction. No untoward by-effects were noted in any of the cases treated.

UYLDERT, I. E. The assay of cortical hormone. *To appear in this Journal.*

The assay of adrenal cortical hormone is described. The assay is performed in healthy dogs of mongrel varieties, 1–2 years old and is based upon clinical symptoms, mainly appetite and body-temperature.

A period of 4 days is sufficient for testing one dose of an extract. It is necessary to determine the limit dose in a large number of dogs and to check this dose with the limit dose of a known standard extract. Waterbalance experiments are of little value for the assay, though from large numbers of estimations it is concluded that in adrenal-insufficiency fluid excretion is increased in relation to fluid intake.

UYLDERT, I. E., J. THOMASSEN AND L. WATERMAN. Contribution to the examination of the blood of normal and adrenalectomized dogs. *To appear in this Journal.*

The blood of dogs before and after adrenalectomy is examined for potassium and sodium amount in the blood plasma, blood sugar and N.P.N. in the blood. Blood samples were taken 15 to 16 hours after the last meal. The administration by mouth of 0.3 gm. of sodium chloride per kg. of bodyweight, 15 hours before the taking of the blood sample, has no effect upon the investigated substances. No change was found after the removal of one adrenal. After removal of both adrenals a significant change was found in the above mentioned substances. However, between adrenalectomized good clinical condition and various stages of insufficiency no sharp difference can be observed from changes in blood chemical substances. Among the first symptoms of insufficiency are to be reckoned an increased amount of N.P.N. and a decreased amount of sugar in the blood and increased potassium and a decreased sodium content in the plasma. For the practical assay of cortin, however, the estimation of these substances is of lesser importance. From the clinical symptoms of appetite and body temperature alone various degrees of insufficiency can be sharply stated.

GONADS

COLE, H. H., AND G. H. HART. The effect of pregnancy and lactation on growth in the rat. *Am. J. Physiol.* 123: 589. 1938.

Although the genetic constitution of an animal is predominant in the determination of ultimate body size, pregnancy is an important factor provided the animal has access to sufficient amounts of an adequate diet (McCollum diet I ad libitum and bi-weekly fresh alfalfa). Statistical data on 51 rats with litter-mate controls presented in tabular and graphical form establish the following conclusions. *a*), Pregnancy stimulates skeletal and tissue growth in the rat beyond that of non-bred litter-mate controls. Precociously mature females (gonadotropic hormone) accentuate this statement. *b*), Excess growth is presumably dependent upon an increased food consumption manifested by the second day after conception. *c*), It is postulated that the copulatory act initiates a nervous stimulation to and increased secretion of hormone from the anterior pituitary.—H. J. Spoor.

COURRIER, R., AND G. GROS. Action of testosterone propionate on the uterus (Influence du propionate de testostérone sur l'utérus). *Compt. rend. Soc. de biol.* 127: 921. 1938.

Immature and adult cats, castrated or normal, were injected daily for 7 to 15 days with 5 to 10 mg. of testosterone propionate (some follicular hormone was administered together with the androgen or before). Uterine hypertrophy was induced; the endometrium in some instances was that seen in the follicular phase of a cycle and in others it was typical of the luteal phase. There was no effect on the vagina. Daily doses of 5 mg. of testosterone propionate failed to interrupt pregnancy in rabbits, but 10 mg. daily did terminate pregnancy. Unlike progesterone, testosterone propionate in daily doses of 10 mg. was incapable of causing successful nidation of zygotes in rabbits; moreover, such treatment in early pregnancy led to atrophy of corpora lutea.—E. Cutuly.

COURRIER, R., AND R. KEHL. Functional correlations of the two ovarian hormones (Sur les corrélations fonctionelles des deux hormones de l'ovaire). *Compt. rend. Soc. de biol.* 127: 140. 1938.

In rabbits a progestational endometrium under the influence of progesterone will eventually undergo involution despite continued treatment with that hormone. Evidence is presented that such endometrial involution is retarded but not prevented by simultaneous injections of estradiol and progesterone.—E. Cutuly.

CRUVEILHIER, L., J. HAGUENAU, G. THIEULIN AND C. VIALA. Influence of gondatropic hormone on the development of the Shope tumor in rabbits (Influence de l'hormone gonadotrope sur l'évolution de la tumeur de Shope du lapin). *Compt. rend. Soc. de biol.* 127: 485. 1938.

Adult female rabbits inoculated with a papilloma were separated into two divisions: one a control and one injected 3 times at intervals of 48 hr. with 250 I.U. of folliculin. The folliculin-injected animals were further subdivided into 3 groups. Each of the 3 groups was injected with a different substance, starting 48 hrs. after the last injection of folliculin. Group one received whole anterior beef pituitary extracts 4 times at intervals of 4 days (1 dose = 250 mg. fresh gland). Group 2 received 8 injections of prolactin at intervals of 2 days (1 dose = 1 mg. fresh gland). Group 3 received 5 injections of PU extract at intervals of 3 days (each dose = ½ RB.U.). Tumors developed in all of the groups, with one exception. In group 3 tumors had begun to develop but were caused to disappear by PU.—E. Cutuly.

DANTCHAKOFF, V. Male hormone responsible for development or malformation of a penis in mammals (L'hormone mâle à la base de l'édification du pénis et ses malformations chez les mammifères). *Compt. rend. Soc. de biol.* 127: 674. 1938.

Injections of testosterone were made directly into the uteri of pregnant guinea pigs. Genetically female offspring of these animals showed varying degrees of genital masculinization; in some a well formed penis developed, while in others this structure showed various deformities.—E. Cutuly.

DRANESLEY, R., AND A. S. PARKES. Biological properties of new derivatives of testosterone. *Biochem. J.* 31: 1161. 1937.

Tested on either capons or castrated rats, the recently prepared testosterone oxime and oxime propionate, in comparison with testosterone and testosterone propionate, are only slightly active. The diacetate of the enolic form of testosterone shows activity. The activity of the diacetate of the enolic form of testosterone is similar to that of testosterone 17-monoacetate.—*Physiol. Abst.*

DE WIT, J. J. D. A new test for the qualitative and quantitative investigation of the corpus luteum hormone (Ein neuer Test zum qualitativen und quantitativen Nachweis des Corpus Luteum Hormones). *Klin. Wchnschr.* 17: 660. 1938.

The author proposes the ovipositor reaction in the bitterling for the assay of corpus luteum hormone. A maximum effect is obtained in 5 hr., and the response curve is very steep for quantities between 4 and 20γ. Estrogens were without effect in this period of time, and although androgens produce a similar reaction, about 50 times as much material is required. The test animals are cheap and may be used repeatedly every few days.—M. H. F.

DE WIT, J. J. D. The reaction of the male and female bitterling to some of the pure sex hormones (Die Reaktion des weiblichen und mannlichen Bitterlings auf einige reine sexual Hormone). *Klin. Wchnschr.* 17: 377. 1938.

Androgens were without effect on the coloring reaction of the male bitterling. When injected into the female, however, they produced increase in length of the ovipositor, the maximum being reached in 18 to 25 hr. When the preparations were added to the aquarium water the latent period was reduced to 6 hr. for the maximum effect. Progesterone behaved qualitatively like the androgens. Very large amounts of estrogens produced a slight reaction, but even when added to the aquarium water the latent period was over 24 hr.—M. H. F.

DORFF, G. B. Intra-abdominal cryptorchidism treated with gonadotropic substance. Observations in a series of twelve prepuberal boys. *J.A.M.A.* 110: 1799. 1938.

With the gonadotropic principle from the urine of pregnant women, the author treated 12 prepuberal boys, 11 presenting unilateral cryptorchidism and 1 bilateral cryptorchidism. The patients were from 3 yr. and 4 mo. to 11 yr. and 8 mo. of age. Doses in most cases were large, and given intensively over extended periods. Two of the boys showed no response. In 9 descent occurred. When impediments such as marked hydrocele accompanied intra-abdominal cryptorchidism and existed before treatment, endocrine therapy caused the intra-abdominal testicle to descend from the abdomen but it did not correct the hydrocele. The existing hypoplastic gonad and external genitals were developed through the use of gonadotropic substance to the point of apparent normality. The only seemingly adverse effect was the precocious production of suprapubic hair in some of the younger boys.—R. G. H.

DWORZAK, H., AND A. BERNDT. The question of the constancy of duration of function of the corpus luteum (Zur Frage der Konstanz der Funktionsdauer des Corpus Luteum). *Zentralbl. f. Gynäk.* 62: 2200. 1938.

To estend the Knaus concept of the duration of the activity of the rabbit corpus luteum these authors have studied two varieties of rabbits, one large and the other small. The animals were made pseudo-pregnant at definite times, and under ether uterine strips were removed and tested for sensitivity to posterior pituitary extract. If negative they were tested with epinephrine. The first appearance of pituitary sensitivity marks the earliest end of progestin function, the latest failure followed by sensitivity marks the longest function. Strips were tested at about 4-hr. intervals from a given rabbit. The results indicate a difference between varieties, 346 to 358 hr. activity in the larger animals, 363 to 370 hr. in the smaller. The gross appearance of the corpora lutea changed from orange yellow to pale yellow at the time when activity disappeared. This is believed to indicate decreased circulation.—E. L. S.

EIDELSBERG, J. The male sex hormone. *Med. Clin. N. America.* 22: 1537. 1938.

This contribution consists essentially of a description of 9 cases of hypogonadism in males, all treated with testosterone propionate (oreton) with considerable success.—I. B.

FINCH, J. W. Etiology of nausea and vomiting of pregnancy: preliminary report. J.A.M.A. 111: 1368. 1938.

The author states that nausea and vomiting of pregnancy develop at the same time at which the corpus luteum of pregnancy reaches an appreciable size. The symptoms disappear at about the time the gland is known to begin retrogressive changes. In a series of patients with nausea and vomiting of pregnancy in varying degrees when injected intradermally with from 0.02 to 0.03 cc. of progestin in oil a cutaneous reaction develops directly proportional to the severity of the symptoms. A control series of patients who were not nauseated and in the pregnant state gave negative cutaneous reactions when tested in the same manner. Patients treated with subcutaneous corpus luteum extract and progestin along the lines of allergic desensitization were gradually relieved of their symptoms. A high percentage ef the patients with nausea and vomiting of pregnancy either had other diseases of allergy or gave a family history of allergy. Intradermal testing may determine, even before pregnancy, whether a patient will or will not be nauseated when pregnant by determining whether or not she is sensitive to progestin.—Abstract service J.A.M.A.

FLAKS, J., AND A. BER. Influence of tumors on response to gonadotropic hormone (Influence des tumeurs sur la réaction de l'hormone gonadotrope). *Compt. rend. Soc. de biol.* 127: 1066. 1938.

Gonadotropic hormone in quantities sufficient to elicit a luteal response in normal mice failed to stimulate the ovaries of mice bearing large tumors (Ehrlich sarcoma). Studies indicated that the tumors neither absorbed nor inactivated the gonadotropic hormone. It was believed that the tumors produced a toxic substance which acted antagonistically to the gonad-stimulating hormone.—E. Cutuly.

GALSTOFF, P. S. Physiology of reproduction of Ostrea virginica. Stimulation of spawning in the female oyster. *Biol. Bull.* 75: 286. 1938.

Physiologically ripe females of *Ostrea virginica* were stimulated to spawning by a sudden rise of temperature from 20° to 32°–35°C. or the presence of live sperm. The latest period for stimulation by sperm in 101 experiments varied from 6 to 38 min. with an average of 16.2. Minimum dose of sperm was found to be 0.2 to 0.3 cc. per 30 l. of water. The active principle of sperm was insoluble in water and soluble in alcohol and benzene. It is inactivated by heating at a temperature of 54° to 57° C.—E. L.

KOBER, S., The colorimetric estimation of the oestrogenic hormones: II, oestrone. *Biochem. J.* 32: 357. 1938.

Beta-naphthol-sulphonic acid is now proposed as a reagent for estrone; methods of applying the test to assay of urine samples are discussed.—D. L. T.

LEONARD, S. L., V. SAGER and J. B. HAMILTON, Effect of male hormone upon uterine motility and the uterus. *Proc. Soc. Exper. Biol. & Med.* 37: 362. 1938.

From 10 to 20 mg. of testosterone propionate in a single injection reduced or completely inhibited the motility of the rabbit uterine fistula in each of several females. Histologic study of the uterus of these animals and of others injected with testosterone after castration indicated definite evidence of progestational proliferation, thus confirming the views of Klein and Parkes.—M. H. F.

LYON, R. A., and W. M. ALLEN, Duration of sensitivity of the endometrium during lactation in the rat. *Am. J. Physiol.* 122: 624. 1938.

A series of mature albino female rats was studied in which, at various times during lactation, the right horn of the uterus was stimulated by multiple perforations with a needle, the animal killed 5 days later, and the presence or absence of deciduomata in the uterus determined by macro and micro examination. A total of 58 animals was studied. It was found that endometrial sensitivity did not appear until the 4th day postpartum; i.e., no deciduomata were obtained in animals stimulated on the 1st, 2nd or 3rd postpartum days. This sensitivity persisted until the 16th day, after which it decreased rapidly. These findings indicate that during lactation the corpora lutea may remain functional for at least 16 days. The results are in marked contrast to the fact that during pregnancy, when the corpora are functional to almost the end of gestation, endometrial sensitization is limited to the 4th, 5th, 6th and 7th days at most. The authors believe this difference in sensitivity during pregnancy and lactation is due to the fact that during the latter part of pregnancy the estrogen is high, whereas it is low during lactation.—S. L. Cohen.

MACHT, D. I., Methods of demonstrating the activity of corpus luteum. *Surg. Gynec. & Obst.* 66: 732. 1938.

The corpus luteum through its internal secretion plays an important rôle with regard to the course of pregnancy, and in primates exerts an influence on the menstrual cycle as indicated by gross anatomical and histological changes in gonads and accessory organs. Experimentally, corpus luteum extracts or preparations have shown an inhibitory effect on the estrous cycle of small animals (guinea pigs and mice).

Extracts of corpus luteum prepared with ether, petroleum-ether, acetone, chloroform, and alcohol, showed activity when tested by the various methods. The best clinical results were obtained from water-soluble extracts. It is the author's contention that it is wiser for the clinician to employ a combination of extracts of the whole ovary or of the corpus luteum approximating as closely as possible the normal content of the gland in its natural state.—L. B. S.

MAYO, C. W., and E. O. STRASSMANN, Fertility and sterility after extra-uterine pregnancy. *Surg. Gynec. & Obst.* 67: 46. 1938.

In a period of 10 years 142 patients with ectopic pregnancies were seen at the Mayo Clinic. Of this number 141 were operated upon with no deaths in the series. One patient died in shock before operation was attempted, the total mortality being 0.7%. In this series 55% of the tubal pregnancies occurred on the right side and 45% on the left. Since study of this series revealed the possibility of an intrauterine pregnancy after one ectopic pregnancy is about 10 times larger than the probability of another ectopic pregnancy, conservative surgery was urged in order to preserve fertility.—L. B. S.

MCKEOWN, T., and S. ZUCKERMAN, The suppression of oestrus in the rat during pregnancy and lactation. *Proc. Roy. Soc. London, s.B.* 124: 464. 1938.

Destruction of the corpora lutea by electrocautery, during pseudopregnancy or pregnancy, leads to premature reappearance of estrus except in the latter half of pregnancy; in lactating animals, destruction of corpora lutea does not lead to reappearance of estrus so long as the litters continue to suckle actively. —D. L. T.

MIESCHER, K., C. SCHOLZ and E. TSCHOPP, The activation of female sex hormones: I, oestrone and its esters. *Biochem. J.* 32: 141. 1938.

The activity of the acetate and propionate of estrone in producing estrus and causing uterine growth in rats is little different from that of the free hormone; but higher esters, up to the stearate, show increased threshold value and prolongation of effect. The greatest over-all efficiency is obtained with the octanoate (caprylate).—D. L. T.

MIESCHER, K., C. SCHOLZ and E. TSCHOPP, The activation of female sex hormones: II, alpha-oestradiol and its di-esters. *Biochem. J.* 32: 725. 1938.

Estradiol dipropionate, didecanoate, and dipalmitate have much greater and more prolonged activity in inducing estrus and uterine growth in rats than free estradiol, or than estrone and its most active esters. —D. L. T.

MILLER, A. G., The fertile period in practice. A five-year clinical study. *Surg. Gynec. & Obst.* 66: 723. 1938.

Of this 5-year study 480 women form the basis. Each woman was required to present a written record of the beginning dates of the last 6 menstruations, and each was given a thorough physical examination which included a search for pelvic pathology. In the author's opinion, the weeding out of abnormal cases accounted for the gratifying results obtained. The study of the menstrual cycles in this series revealed a law of recurrence which he expressed as follows, "Barring accident or disease, the menstrual cycle of normal women varies in length continually between a certain maximum and minimum number of days." It was found that ovulation had a definite time relationship to the next succeeding menstruation and regardless of the rhythm in the menstrual cycle this relationship does not change. In this large series not a single pregnancy resulted from cohabitation outside of the fertile periods established for the various patients, therefore leading the author to affirm the biologic law of the existence of definite periods of physiologic sterility and fertility.—L. B. S.

MISHELL, D. R., Familial intersexuality. A report of three unusual cases. *Am. J. Obst. & Gynec.* 35: 960. 1938.

Of 3 sisters, 2 were found at operation to have male gonads, without ovaries, tubes, or uterus. The third presented similar facts on clinical examination. All had estrogen in the urine, disappearing after operation, when gonadotropic substance was found.—E. L. S.

HYPOPHYSIS

ATKINSON, F. R. B. Acromegaly, Description of Papers Reported in 1935, 1936, 1937. *Endokrinol.* 20: 245. 1938.

A survey of papers published since the author's résumé of papers of 1931–35 which was published in *Endokrinology* in 1936.

Berblinger, W. Post-traumatic inflammatory fibrous atrophy of the neurohypophysis with diabetes insipidus (Diabetes insipidus bei entzündlich-fibröser Atrophie der Neurohypophyse nach Unfall.) *Endokrinol.* 20: 189. 1938.

Autopsy of a case of diabetes insipidus is reported. The neural lobe of the hypophysis showed evidences of an inflammatory reaction which had terminated in fibrous atrophy. The other lobes of the hypophysis were normal in appearance. Although the neural lobe was atrophic, the supraoptic and tuberal nuclei were normal in appearance. Regressive changes were seen in the nucleus mamilloinfundibularis. The author concludes that diabetes insipidus in man can follow a marked reduction or complete interruption of the function of the pars nervosa. For the manifestation of diabetes insipidus it is apparently necessary that the function of the anterior lobe be maintained. It is further stated that the pars intermedia and the pars tuberalis can be completely destroyed without the development of diabetes insipidus. On the other hand in fully developed diabetes insipidus the pars intermedia and pars tuberalis may be found intact.—W. J. A.

Bernard, A. T. Concentration of hypophyseal gonad-stimulants and urinary prolan by ultrafiltration (Concentration des gonado-stimulines hypophysaires et de prolans urinaires par ultrafiltration). *Compt. rend. Soc. de biol.* 127: 644. 1938.

It is asserted that ultrafiltration of urine through cellophane yields a highly concentrated residue of gonadotropic substances while follicular hormone passes into the ultrafiltrate.—E. Cutuly.

Bernstein, M., M. Moore and D. Fishback. Diabetes insipidus as a sign of metastatic involvement of the supraopticohypophyseal system. *Arch. Int. Med.* 62: 604. 1938.

The literature of the subject is reviewed and a case is described. The patient was a man aged 64 who presented the symptoms of diabetes insipidus. The history, the physical examination and the laboratory data gave no definite clues as to the etiology of the disturbed water metabolism. Death occurred 5 weeks after the primary examination, following an attack of hemiplegia. The post-mortem findings revealed a primary bronchogenic carcinoma involving the left lung with metastasis to the serous membranes of the thoracic and abdominal organs and to the brain, cerebellum, hypophysis and infundibulum. It is suggested that in the absence of conclusive evidence as to the etiology in a patient with diabetes insipidus, masked malignancy should be suspected and an effort made to establish the primary source.—Authors' Abstract.

Bruckner, W. J., C. H. Wies and P. H. Lavietes. Anorexia nervosa and pituitary cachexia. *Am. J. M. Sc.* 196: 663. 1938.

Two cases of anorexia nervosa are reported in which the signs of Simmonds' disease were closely simulated. This was especially true of the first case in which there was no apparent psychologic background for the disease. The diagnosis was established by the response to feeding. It was suggested earlier by the obvious psychopathy and physical activity of the second patient, by the age of the patients, by the lack of etiologic factors for pituitary destruction and by the preservation of the breasts. The depression of metabolic processes, disturbances of carbohydrate metabolism and suppression of menses have been shown to have no value in differentiating anorexia nervosa from Simmonds' disease. Loss of pubic and axillary hair, if complete or almost so, suggests Simmonds' disease; sparsity or failure of development of this hair may occur in either condition. It is apparent that no matter how strongly a patient's condition suggests Simmonds' disease skepticism is in order until adequate feeding has been given a fair trial; this is especially true in young girls, in whom anorexia nervosa is common and Simmonds' disease rare.—Authors' abstract (abbreviated).

Flaks, J., I. Himmel and A. Zlotnik. Existence of a hematopoietic hormone in the hypophysis (Sur l'existence d'une hormone hémopoïétique dans l'hypophyse). *Presse méd.* 45: 1261. 1937.

In rats it was found that prolonged oral administration of anterior lobe of the hypophysis produced an increase in the number of reticulocytes and consecutive augmentation in the erythrocytes, with resulting prolonged polyglobulism. The active substance was not found in the deproteinized fraction and was found to be thermostable. The hormone is stated to act directly on the bone marrow and to be without influence on the thyroid. The injection of a quantity of extract corresponding to 0.4 gm. of fresh hypophysis produced after 24 hr. transformation of gray into red marrow. On the basis of clinical observation and the experience described, the authors conclude that the hypophysis has an important physiologic rôle in the regulation of erythropoiesis by means of an hematopoietic hormone which acts on the bone marrow.—From abstract in *Arch. Neurol. & Psychiat.*

Foglia, V. G., and P. Mazzocco. Effect of anterior pituitary extract on liver and muscle fat (Action de l'extrait antéro-hypophysaire sur la graisse hépatique et musculaire). *Compt. rend. Soc. de biol.* 127: 150. 1938.

Diabetogenic alkaline extracts of anterior pituitary administered to dogs increased liver fat and glycogen while pancreatectomy increased liver fat but decreased liver glycogen. The extract caused

a less noticeable rise in muscle fat. Hyperglycemia, glycosuria, ketonuria and ketonemia were elevated by the extract, but not as markedly as after pancreatectomy. Injection of the extract after pancreatectomy enormously increased ketonemia. The extract was effective in the absence of the thyroid, adrenal medulla and the splanchnic nerves.—E. Cutuly.

Friere, M. A. Effect of estrone on the gonadotropic potency of the hypophysis of the toad, *Bufo arenarum* (Action de l'oestrone sur le pouvoir gonadotropique de l'hypophyse du crapaud *Bufo arenarum*). *Compt. rend. Soc. de biol.* 127: 560. 1938.

The pituitary glands of 20 toads injected daily for 14 to 20 days with 60 I.U. of estrone did not show decreased gonadotropic power as evidenced by their ability to induce ovulation when implanted into other female toads.—E. Cutuly.

Gatz, A. J. The cytological relationship between the hypophysis and the germinal epithelium of the testis. *Anat. Rec.* 70: 619. 1938.

The loss of the normal germinal epithelium of the testes, through gonadectomy, vasectomy, artificial cryptorchidism, x-radiation, or senility, produces similar modifications in the anterior lobe of the rat hypophysis. Measurements show that the acidophiles and their cell components decrease in size, but other cytological modifications were not observed. The basophiles, which become more numerous, are doubtlessly derived from the chromophobe cells. The loss of the normal germinal epithelium stimulates the differentiation of the chromophobes into basophiles. Two months after the loss of the normal seminiferous epithelium, the basophiles may be arbitrarily separated into three groups of cells; normal appearing basophiles, enlarged basophiles, and vacuolated basophiles. The Golgi material of the basophiles increases in size immediately after the loss of the germinal epithelium. An active secretory cycle in the basophiles is shown by the presence of numerous secretion droplets, surrounded by osmiophilic girdles, in the central or peripheral portions of the Golgi material. The degenerate seminiferous epithelium may have some inhibitory influence upon the Golgi substance of the basophiles, since it was largest in the animals which had been gonadectomized. The large vacuolated cells, which originated from the basophiles 2 months after the loss of the normal germinal epithelium, may be appropriately termed 'sterility cells' in the rat. These cells apparently are the reservoirs of the basophilic secretion.—Authors' Abstract, Wistar Bib. Service.

González, R. Influence of the hypophysis in renal compensatory hypertrophy (Influencia de la hipófisis sobre la hipertrofia renal compensadora). *Rev. Soc. argent. de Biol.* 14: 173. 1938.

Compensatory hypertrophy of the remaining kidney after removal of the other is considerably reduced in hypophysectomized toads, from $44 \pm 4\%$ in 10 to 15 days and $58 \pm 7\%$ in 20 days in normal controls to $22 \pm 3\%$ and $23 \pm 6\%$ in the operated animals. Implantation of the whole hypophysis in the hypophysectomized permitted an increase of $100 \pm 17\%$ in the weight of the remaining kidney. Implantation of anterior lobe gave an increase of $63 \pm 7\%$, and of the posterior lobe one of $70 \pm 8\%$.—J. T. L.

Houssay, B. A., and A. Biasotti. Diabetogenic activity of various hypophyseal hormones (Acción diabetógena de diversas hormonas hipofisarias). *Rev. Soc. Argent. de Biol.* 14: 297. 1938.

The existence of diabetogenic activity in an extract cannot be maintained unless the fasting blood sugar is raised at least to 160 mg. %. Transitory hyperglycemias should not be taken into account. Subtotal extirpation of the pancreas (leaving 3–4 g. pancreatic tissue), increases sensitivity of dogs towards the diabetogenic action of A.P. extracts; very small doses are effective and prolonged treatment produces permanent diabetes. Occasionally extracts of other tissue produce diabetes, therefore this type of animal does not react in a strictly specific way as does the intact animal. The hypophysectomized dog with subtotal pancreatectomy is very sensitive to A.P. extract; 20 to 40 mg. per kg. per day fresh bovine A.P. intraperitoneally produces an intense diabetes with blood sugar levels of 180 to 250 mg. %. In 1 to 3 days after suppression of the injections the blood sugar returns to normal. In this type of dog and in the hypophysectomized pancreatectomized toad Riddle's prolactin (with and without adrenotropic activity) and Collip's adrenotropic extract had no diabetogenic action. FSH prepared according to Fevold and Hisaw had diabetogenic activity in the toad; the LH had slight diabetogenic activity in the toad but none in the dog. Praephyson a commercial A.P. extract (weak gonadotropic, adrenotropic and cetogenic activity, moderately strong thyrotropic activity) had no diabetogenic activity in the normal dog and a slight diabetogenic activity in the subtotally pancreatectomized hypophysectomized dog.—J. T. L.

Houssay, B. A., and A. Biasotti. Diabetogenic activity of anterior pituitary extract in adrenalectomized dogs (Acción diabetógena ánterohipofisaria en perros sin suprarrenales). *Rev. Soc. argent. de Biol.* 14: 308. 1938.

Anterior pituitary diabetogenic extracts produced diabetes in toads after removal of the pancreas and the acrenals, the hypophysis remaining intact or having been extirpated. In adrenalectomized dogs,

with subtotal pancreatectomy, maintained in good condition by an appropriate saline diet and daily injections of cortico-adrenal extract, the blood sugar remained normal until a diabetogenic A.P. extract was injected intraperitoneally, when diabetes occurred. Therefore the diabetogenic activity of the extract is not exercised through the adrenals.—J. T. L.

HUMBERD, C. D. Giantism: Report of a case. *South. M. J.* 31: 998. 1938.

An acromegalic giant, 22½ years old and 7 ft. 6 in. tall, is described. Careful measurements and physical findings are given. The noteworthy feature was that, aside from his size, he was physically and mentally normal.—J. C. D.

JACOBSON, A. P. Comparison of the chemical composition of mammary secretions in rabbits during normal lactation and after administration of prolactin (Vergleich zwischen der chemischen Zusammensetzung des Brustdrusensekrets beim Kaninchen während der normalen Laktation und nach Prolaktinverabreichung). *Skand. Arch. f. Physiol.* 79: 97. 1938.

The composition of the milk of 3 normal lactating rabbits was compared with that obtained by inducing lactation in 6 multiparous rabbits. The milk of both sources had the same fat and total nitrogen contents and showed no significant differences in lactose contents.—A. G.

LAHR, E. L., AND O. RIDDLE. Proliferation of crop-sac epithelium in incubating and in prolactin-injected pigeons studied with the colchicine method. *Am. J. Physiol.* 123: 614. 1938.

Optimum dosage of colchicine sufficient to arrest all dividing cells and yet low enough to permit all such cells eventually to complete their mitoses has been established for 400 to 500-gram pigeons at 0.6 mg. per pectoral muscle injection. Utilizing this technique the authors have presented data, two graphs and eight tissue section (crop gland epithelium) photographs to establish; *a*), immediate response of crop-sac epithelium to prolactin injection (60 U), showing a maximal stimulus at 2 hr. and minimal at 10 to 12 hr., obtaining stimulation by injections at 8-hr. intervals; *b*), similar changes during the onset of incubation indicate that prolactin from the animals' own pituitary plays a demonstrable rôle in the cyclic maternal behavior of pigeons.—H. J. Spoor.

LI, R. C. The relationship between dosage of prolan and weight response of testes and accessory sex organs in the immature rat. *Chinese J. Physiol.* 13: 323. 1938.

The dose-response relationship of the testes and the accessory male sex organs was investigated by weighing the fresh organs of immature albino rats receiving graded doses of a highly purified I.G.F. prolan. One hundred and twenty-two rats belonging to 28 litters were used. One animal of each litter served as control, the others being injected with 0.01, 0.02, 0.04, 0.08, 0.14 and 0.20 mg. of prolan per rat. The seminal vesicles, coagulating glands (anterior lobe of prostate) and the prostate showed a more proportionate increase of weight with increasing dosage of prolan than did the testes and epididymis. Therefore, while all may be used as criteria in the qualitative estimation of the hormone, the first three are better for quantitative analysis.—Gertrude Russack.

PANCREAS

BAUDOUIN, A., J. LEWIN AND E. AZÉRAD. Slow and continuous insulin injections in diabetes (Injections lentes et continues d'insuline chez les diabétiques). *Presse méd.* 46: 729. 1938.

The minimal dose producing a fall in blood sugar in the dog is 0.05 U per hr. per kg. if given intravenously and 0.01 U intra-arterially. The corresponding dose in man is 0.01 to 0.02 U intarvenously. It is calculated that the total insulin secreted by a man of 60 kg. during 1 hr. is 6γ. Diabetic patients with a glucemia of 200 to 300 mg. required 0.2 to 0.3 U per kg. per hr. in order to lower the blood sugar to 100 mg. within 3 hr. This level, once attained, could be kept up with 0.01 U. While the insulin has an immediate effect on the blood sugar and its excretion in the urine, the acetonuria persists. The continuous injection gave excellent results in critical diabetic conditions.—A. E. M.

BAUDOUIN, A., J. LEWIN AND E. AZÉRAD. Continuous injections of insulin in diabetics. Dose necessary to restore normal glycemia (Injections continues d'insuline chez les diabétiques. Dose nécessaire pour ramener la glycémia à la normale). *Compt. rend. Soc. de biol.* 127: 378. 1938.

BAUDOUIN, A., E. AZÉRAD AND J. LEWIN. Continuous intravenous injections of insulin. Maintenance dose in 3 diabetics (Injections intraveineuse continues d'insuline. Recherche de la dose d'entretien chez trois diabétiques). *Compt. rend. Soc. de biol.* 127: 380. 1938.

In the first report are given the details of a technic by which the dose of insulin necessary to maintain a constant level of blood sugar was determined in 3 diabetics (second report). Continuous intravenous injections of a solution containing insulin, starting with about 0.3 U, were made over a period of 3 hours and then a rest period of ½ to 1 hour was allowed. This procedure was repeated 1 to 3 times, each

time with the dose of insulin decreased, until the maintenance dose was found. This dose was found to be 0.01 U in 2 of the patients and 0.1 to 0.3 U per kg. per hour in the third. Addition of glucose to the insulin solution caused a rapid disappearance of ketonuria.—E. Cutuly.

Cristol, P., L. Hedon, A. Loubatières and P. Monnier. Increase in glycogen and decrease in lipoids in the liver in normal fasting dogs after repeated weak doses of insulin (Enrichissement en glycogène et appauvrissement en lipides du foie, chez le chien normal à jeun, sous l'influence de doses d'insulin faibles et répétées). *Compt. rend. Soc. de biol.* 127: 23. 1938.

Biopsy fragments of liver and thigh muscle in 3 dogs were assayed before and after subcutaneous injections of insulin. Insulin was given repeatedly over periods of ½ to 25 hr. in doses which were small but adequate to maintain marked hypoglycemia. Such treatment caused a decided increase in liver glycogen and a possible decrease in liver lipoids.—E. Cutuly.

De, P., and S. Bhattacharyya. Glycolysis in drawn blood in twenty-four hours. *Indian J. M. Research.* 25: 933. 1938.

De and Bhattacharyya compared the hourly rate of glycolysis of blood from normal and diabetic persons. Glycolysis takes place in normal blood (0.1% solution of potassium oxalate being used as an anticoagulant) at a rate of from 3.83 to 6.5 mg. (total range from 2 to 11 mg.) an hour per 100 cc. of blood, giving an average rate of 5.56 mg. an hour. The calculated average velocity of percentile glycolysis is 6.05 mg. In diabetic blood in oxalated samples glycolysis proceeds at a rate of from 4 to 16.6 mg. an hour per 100 cc. of blood (total range 0 to 36 mg. an hour), giving an average rate of 11.06 mg. an hour. The calculated average velocity of percentile glycolysis in diabetic blood is found to be 4.61 mg. The average hourly velocity of percentile glycolysis in diabetic blood shows a depression of about 23.8% from normal blood.—Abstract from *J.A.M.A.*

Dorst, S. Chronic hypoglycemia. A problem in carbohydrate metabolism. *Am. J. M. Sc.* 196: 688. 1938.

It was noted by the author that some undernourished patients gained weight under insulin treatment but many did not. In a search for prognostic criteria an investigation was made of the carbohydrate metabolism. A group of patients were discovered most of whom were underweight and who showed low, flat glucose-tolerance curves. The curve served to differentiate them from subjects of hyperinsulinism. When treated with small doses of insulin a number of the patients with chronic hypoglycemia showed marked clinical improvement, an increase in sense of well-being, gain in weight, and increased appetite; concomitant with this improvement the glucose tolerance curve returned to the normal configuration. The precise action of the insulin in effecting this change is not at present understood. The possibility is suggested that the demobilization of hepatic glycogen is in some way inhibited.—R. G. H.

Florentin, P., and P. Grognot. Experimental changes in the endocrine pancreas produced by vagotonine (Modifications expérimentales du pancréas endocrine sur l'influence de la vagotonine). *Compt. rend. Soc. de biol.* 127: 1082. 1938.

Insulin-free vagotonine produced in guinea pigs a rapid hyperplasia of pancreatic islet tissue which subsided after about 5 days.—E. Cutuly.

Himwich, H. E., J. F. Fazekas and S. J. Martin. The effect of bilateral ligation of the lumbo-adrenal veins on the course of pancreatic diabetes. *Am. J. Physiol.* 123: 725. 1938.

Thirteen dogs and 20 cats subjected to bilateral ligation of the lumbo-adrenal veins and total pancreatectomy at one operation survived an average of 17.5 and 19.0 days respectively, in the absence of any sort of therapy (cortin, insulin, added NaCl). Ameliorated diabetes was further evidenced by a lower blood sugar level, decreased glycosuria, nitrogen excretion, ketonuria and D/N. Blood fat was usually below the diabetic level. Carbohydrate balance studies revealed an average retention of 10 g. glucose. This glucose was probably oxidized as indicated by a total R.Q. of 0.79. However, in only 2 out of 14 observations was a significant rise in R.Q. observed after glucose ingestion. In many instances (5 out of 7) the total base content of the serum was within normal limits. Thus the change in carbohydrate metabolism does not appear to be secondary to that of inorganic metabolism.—K. A. Brownell.

PARATHYROID

Albright, F., H. W. Sulkowitch and E. Bloomberg. Hyperparathyroidism due to idiopathic hypertrophy (hyperplasia?) of parathyroid tissue. *Arch. Int. Med.* 62: 199. 1938.

Six cases of hyperparathyroidism with what has previously been termed primary hyperplasia of the parathyroid glands are reviewed. It is pointed out that the pathologic condition of the parathyroid glands is histologically dissimilar from that of cases of undoubted hyperplasia of parathyroid tissue, that it has not yet been shown that the enormous enlargement of the glands in this condition (about 30 to 100 times) cannot be explained by hypertrophy of the cells and that the condition may be a disorder of

hormone production rather than hyperplasia. The studies showed that all the glands from all 6 patients on all occasions revealed a similar histologic picture; it is suggested that the tissue change is an 'all-or-none' one. A distinct correlation was observed between the weight of the parathyroid tissue and the degree of hyperparathyroidism; this was in marked contrast to the situation in cases of parathyroid adenoma. Evidence is presented that the underlying cause of the changes in the parathyroid glands is chronic; in 1 case the condition had existed for at least 10 yr. There was little if any evidence of a tendency for the parathyroid tissue left in place after partial resection to regenerate, so the condition is apparently amenable to permanent surgical cure. The optimum amount of tissue to be left at operation has not yet been determined, but anything less than 400 mg. is probably not too much. No evidence has been obtained to confirm the hypothesis that the condition is secondary to overactivity of some pituitary hormone.—I. B.

Bastenie, P., and S. Zylberszac. Evidence of hormonal stimulation revealed by colchicine. Stimulation of the parathyroid (Mise en évidence de stimulations hormonales par la colchicine. Stimulation de la parathyroïde). *Compt. rend. Soc. de Biol.* 127: 822. 1938.

Castrated rats injected with follicular hormone and progesterone were treated with colchicine. Sections of their parathyroid glands revealed vast numbers of mitotic figures as compared with the few figures observed when colchicine was not given.—E. Cutuly.

Cope, O., and G. A. Donaldson. Thyroid and parathyroid in calcium and phosphorus metabolism. *J. Clin. Investigation*, 16: 329. 1938.

The Ca and P metabolism is here studied in a patient with recurrent thyrotoxicosis and postoperative parathyroid tetany. When the metabolic rate was maintained within normal limits with I medication, the Ca and P ratios were characteristic of parathyroid tetany. In the presence of hyperthyroidism a marked increase in the negative balance of Ca and P was found, even though signs of diminished parathyroid activity continued. Increased thyroid activity was followed by a rise of the blood serum Ca level and a decrease in the tetany. With decrease in thyroid function the opposite took place. An acute infection of bronchi was associated with an increase in tetany and a remission of the thyrotoxicosis.—From Abstract in *J. Nerv. & Ment. Dis.*

Franck, S., and N. Hjerrild. Recurring osteitis fibrosa with parathyroid adenoma. *Hospitalstidende* 80: 1117.

These observers show from a patient with generalized osteitis fibrosa with parathyroid adenoma and diffuse hyperplasia of basophil elements in the anterior lobe of the pituitary that there was recurrence about 4 years after removal of an adenoma of the parathyroid. Grave changes in different endocrine glands were present at autopsy, especially an adenoma in the left parathyroid, together with diffuse hyperplasia of the basophil cells of the anterior lobe of the pituitary is probably produced by its basophil elements is one of the hypotheses presented.—From abstract in *J. Nerv. & Ment. Dis.*

THYMUS

Chiodi, H. Prepuberal castration on the thymus and lymphatic nodes of male albino rats (Acción de la castración prepuberal sobre el timo y ganglios linfáticos de ratas albinas machos). *Rev. Soc. argent. de Biol.* 14: 74. 1938.

Male rats from an inbred stock were castrated at 30 days of age. They were killed at different ages up to 250 days, the thymus and submaxillary, cervical and axillary lymphatic nodes were weighed and compared with those of a litter-mate control who had lived with the castrate. Altogether 92 castrates and 92 controls were studied. The absolute weight of the thymus increased up to 100 days in the operated and up to 80 days in the controls; then a rapid decrease was seen up 120 days in both groups; afterwards a gradual decrease took place. The thymus weight per 100 g. body weight, decreased in both groups from the 45th day onward, more rapidly up to 120 days, and gradually thereafter. The curve corresponding to the castrates was parallel, but at a higher level than that corresponding to the controls. The castrates had a slightly greater proportion of cortex than the controls of that age, but no significant differences were observed between both groups in respect of histological aspect, or intensity of involution. This last began a little later, but was at first more marked in the castrates. It is deduced that the gonads play no significant rôle in the physiologic involution of male albino rats' thymus. Prepuberal castration produced a slight increase in weight in the lymph nodes studied; involution started in both groups at 160 days of age, later therefore than in the thymus.—J. T. L.

Chiodi, H. Prepuberal castration on the thymus and lymphatic nodes of female albino rats (Acción de la castración prepuberal sobre el timo y ganglios linfáticos de ratas albinas hembras). *Rev. Soc. argent. de Biol.* 14: 222. 1938.

Ovariectomy was performed at 30 days of age; the thymus observed between 45 and 200 days of age was found to weigh more than in litter-mate controls. The increase in weight was due to thymic tis-

sue. The age: weight curve is similar in thymectomized and controls, but at a higher level in the former. The greatest absolute weight was observed at 60 days in both groups, after that a rapid decrease occurred up to 120 days and a more gradual one from then on. Castration did not influence the rapidity or degree of this decline in weight, therefore the gonads do not play a part in the physiological involution of the thymus; but they are an inhibiting factor in the general growth of this organ. The weight of the submaxillary, cervical and axillary ganglia was greater in thymectomized animals than in the controls, the age: weight curve was similar in both, involution began at 160 days of age.—J. T. L.

CHIODI, H. Effect of sexual hormones on the thymus (Acción de las hormonas sexuales sobre el timo). *Rev. Soc. Argent. de Biol.* 14: 309. 1938.

Male and female rats were castrated at 35 to 45 days of age; at 80 days of age they were injected subcutaneously 0.5 and 1.5 mg. testosterone propionate daily for 11 days. Others received 10 U estrone during 6 days. They all showed a considerably smaller thymus than controls injected with oil. The reduction in weight of the thymus was proportionate to the dose of hormone given, but did not show any difference in relation to the sex of the animal. Normal animals of both sexes treated with the same doses of male and female hormones, also showed a reduction in thymus weight. Histological study showed a considerable destruction of thymocytes, an almost complete loss of the epithelial cells and an increase in connective and adipose tissue. The effect of the female and male hormones was apparently identical. These alterations can not be considered as identical to those caused by accidental involution.—J. T. L.

CHIODI, H. Effect of thyroidectomy and adrenalectomy on the thymus of castrated albino rats (Acción de la tiroidectomía y suprarrenalectomía sobre el timo de ratas albinas castradas). *Rev. Soc. argent. de Biol.* 14: 322. 1938.

Male and female rats were castrated at 30 to 40 days of age, the thyroid was removed at 50 to 70 days of age and the animals killed at varying intervals up to 2 mo. after thyroidectomy. The absolute and relative weight of the thymus of thyroidectomized rats was considerably less than that of operated but not thyroidectomized controls. When thyroid regeneration, due to incomplete extirpation, occurred, the thymus weight approximated the normal. Adrenalectomy performed at 80 and 120 days of age in castrated rats of both sexes did not influence significantly the weight of the thymus observed 30 days after operation.—J. T. L.

CHIODI, H. Effects of Hanson's thymus extract on growth and development of 5 successive generations of albino rats (Acción de la inyección del extracto de timo (Hanson) sobre el crecimiento y desarrollo de 5 generaciones sucesivas de ratas albinas). *Rev. Soc. argent. de Biol.* 14: 326. 1938.

Rats received an extract of calf thymus prepared weekly according to Hanson's technique and kept frozen, in doses of 1 cc. daily intraperitoneally. The weight at birth and weight curves up to 40 days of life did not vary from that of normal controls in the 5 generations treated with this extract; neither was there any significant difference in the age or opening of the eyes, eruption of teeth, growth of hair, or puberty.—J. T. L.

CHIODI, H. Effect of thymectomy on growth and development of albino rats (Acción de la timectomía sobre el crecimiento y desarrollo de ratas albinas). *Rev. Soc. argent. de biol.* 14: 383. 1938.

The thymus was removed from rats 20 to 30 days old. Litter mates were operated similarly but the thymus left intact. Weight curves up to 250 days showed no significant difference in thymectomized and controls. At 120 and 200 days there were no significant variations in weight of lungs, heart, kidney, liver, internal secretion glands and gonads. There was no alteration in the microscopic aspect of the bone. In 5 successive generations the thymus was removed at an early age, but no variation from the normal was observed in the growth curve, nor in development of teeth, hair growth, opening of eyes or descent of testicles.—J. T. L.

ORNSTEIN, I., AND E. LUCINESCU. Thymectomy and blood calcium (Ablation du thymus et calcémie). *Compt. rend. Soc. de biol.* 127: 1039. 1938.

The blood calcium of dogs surviving thymectomy for 10 to 14 days showed negligible changes when compared with normal values.—E. Cutuly.

TURPIN, R., J. SÉRANE AND J. VALLETTA. Effect of thymectomy on hepatic glutathione in albino rats (De l'influence de la thymectomie sur le glutathion hépatique du rat albinos). *Compt. rend. Soc. de biol.* 127: 96. 1938.

Thymectomy was performed in a series of rats between the ages of 30 and 46 days. Experimental and control animals were killed afterwards at intervals between 4 and 56 days and determinations were made on their total and reduced hepatic glutathione. It was found that after a transient decrease in both

control and operated animals due to the anesthesia (numal), the liver GSH returned to normal levels; and it remained normal in control rats. Thymectomized animals, however, showed a decrease in hepatic GSH after 11 days. This decrease became marked after 31 days, but was not evident after 56 days.—E. Cutuly.

THYROID

ALTHAUSEN T. L., AND M. STOCKHOLM. Influence of the thyroid gland on absorption in the digestive tract. *Am. J. Physiol.* 123: 577. 1938.

Hyperthyroid patients show lowered tolerance for dextrose and galactose. In order to find out whether absorption from the digestive tract is affected in diseases of the thyroid, hyperthyroidism was produced in rats by injections of thyroxin and hypothyroidism by thyroidectomy. Sugars, fats and alanin were fed to postabsorptive animals by stomach tube and an hour later, the rats were killed in order to determine the unabsorbed material in the gastrointestinal tract. The thyroid hormone was shown to increase the absorption of dextrose, galactose, xylose, oleic acid but not alanin. These results are not due to a depletion of carbohydrate, increased metabolism, a rise in the velocity of blood flow, intestinal hyperperistalsis, decreased emptying time of the stomach, or increased permeability of the intestinal mucosa. Observations using phlorhizin suggest that phosphorylation is the important factor in speeding up the absorption. The lowered glucose tolerance in the hyperthyroid patient would be explained by faster intestinal phosphorylation of sugar.—G. C. Ring.

BARBIER, P., AND H. PÉQUIGNOT. Effect of thyroid secretion on the blood levels of cholesterol esters (Influence de la sécrétion thyroïdienne sur le taux dans le sang des esters du cholestérol). *Compt. rend. Soc. de biol.* 127: 111. 1938.

Thyroxin caused a decrease in blood-cholesterol esters in 2 human subjects. Another individual, typically myxedematous, had a high level of cholesterol esters in the blood which was caused to disappear in the course of a few months by daily subcutaneous doses of 1 mg. of thyroxin; the total (free) cholesterol in the blood, however, remained high. Data on 5 hyperthyroid individuals, of whom 3 showed low total blood cholesterol, 1 a normal level, and 1 a high level, indicated a reduced amount of esterified blood cholesterol. Thus it was believed necessary to distinguish in hypercholesterolemias, a hypercholesterolemia with low levels of free cholesterol (hypothyroidism) and in hypocholesterolemias, a hypocholesterolemia with low levels of blood cholesterol esters (hyperthyroidism).—E. Cutuly.

BARROW, S. C. Further observations on intensive radiation of hyperthyroidism. *South. M. J.* 31: 737. 1938.

This is a plea, with the quotation of statistics and the presentation of 4 cases, for the use of x-ray in preference to surgery in hyperthyroidism.—J. C. D.

BIILMAN, G. The blood iodine level, its determination and its relation to the basal metabolism, particularly in myxedema and in Grave's disease (Ueber den Blutjodspiegel seine Bestimmung und seine Beziehung zum Gundumsatz, besonders bei Myxoedem und Morbus Basedowii). *Skandinav Arch. f. Physiol.*, Suppl. No. 12 to vol. 79. 1938.

Normal human blood contains 13γ of I per 100 cc., of which 5γ are bound to protein. The protein-bound I is relatively constant remaining unaffected by prolonged administration of KI. In patients with myxedema the protein-bound I is low returning to normal values after thyroid therapy. In Grave's disease the protein bound iodine is high and sinks as the basal metabolic rate decreases under treatment.—A. G.

BRAM, I. Exophthalmic goiter in patients past age 50. Comments based on a series of 322 cases. *Pennsylvania M. J.* 42: 117. 1938.

A series of 322 cases of Graves' disease in patients between the ages of 50 and 68 are discussed. While the sex incidence in Graves' disease in children is approximately 1 male to 20 females and in young adults 1 male to 5 females, in elderly patients in this series the incidence was approximately 1 male to 2 females. Tachycardia was moderate. Most patients presented a chronic syndrome of several years' duration, with arterial hypertension, auricular fibrillation, precordial distress, shortness of breath, and impending or actual congestive heart failure. Other noteworthy characteristics were only moderately enlargement or normal size of the thyroid; exophthalmos of slight or moderate degree and frequently associated with swelling of the eyelids; tremor constant and coarser than in young patients; usually marked loss in weight, fatigability, and weakness. The B.M.R. was generally moderately elevated and occasionally normal. It is emphasized that in patients presenting dominating heart symptoms and arterial hypertension the circulatory phenomena readily 'mask' the basic causative syndrome. Because of the increased hazard due to inadequate circulatory reserve and advancing years, these cases are poor surgical risks.—Abbreviated Author's Abstract.